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SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

Proceedings of a Conference on COTTONSEED PROTEIN FOR ANIMAL AND MAN

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FOREWORD

These proceedings report the information on cottonseed protein for animal and human use, presented at the Conference on that subject held at the Southern Utilization Research and Development Division in New Orleans, Louisiana. The Conference was sponsored jointly by the Southern Division, the United Nations Children's Fund (UNICEF), and the National Cottonseed Products Association. It was attended by over 40 persons, representing the cottonseed, food and associated industries, Federal, state and foreign research laboratories, and international agencies, as well as staff members of the Southern Utilization Research and Development Division.

The reports presented at the Conference were for the purposes of: reviewing the available information on cottonseed protein in rations for animal and man; defining optimum quality of cottonseed products; considering feasible methods and conditions for producing cottonseed protein of a high nutritive quality; and establishing areas of research necessary to the fostering of broader use of cottonseed protein.

1st Row (left to right):

(1) H. B. Barker, (2) H. L. Wilcke, (3) J. Mauron, (4) M. Womack, (5) J. C. Cowan, (6) H. Gutierrez, (7) H. D. Fincher.

2nd Row (left to right):

(8) T. H. Hopper, (9) W. R. Graham, (10) R. B. Bradfield, (11) N. S. Scrimshaw, (12) M. Milner, (13) W. W. Meinke.

3rd Row (left to right):

(14) A. M. Altschul. (15) R. A. Phelps, (16) W. G. Quinn, (17) J. B. Allison, (18) R. C. Whittington, (19) L. V. Curtin, (20) P. D. Aines, (21) F. H. Smith.

4th Row (left to right):

(22) M. L. Karon, (23) A. R. Kemmerer, (24) G. A. Harper, (25) J. H. Hopper, (26) V. L. Frampton (27) L. A. Hewgill, (28) B. Lopez, (29) W. J. Wolf, (30) E. A. Gastrock, (31) L. E. Kovacs, (32) C. M. Lyman, (33) W. B. Anthony, (34) R. Bressani, (35) B. F. Barrentine, (36) G. Paggi, (37) L. E. Allen.

INTRODUCTORY REMARKS

Mr. A. L. Ward, Honorary Chairman, reviewed the historical background of the development of cottonseed meal as a feed and foodstuff.

Dr. G. E. Goheen, Acting Director, Southern Division, in welcoming the members of the Conference, gave a brief description of the organization of the Division.

Mr. Layton E. Allen, Senior Engineer, Food Conservation Division, UNICEF, told of the interest of UNICEF in cottonseed meal as a supplement for protein deficient foods.

Mr. Garlon A. Harper, Director, Research and Educational Division, National Cottonseed Products Association, briefly reviewed current research on cottonseed meal.

THE DETERMINATION OF THE NUTRITIVE VALUE OF COTTONSEED FLOUR[1]

by

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This study of the nutritive value of cottonseed flour was divided into two parts: (1) a short-term feeding experiment lasting 2 months and (2) a long-term study on reproduction and lactation lasting approximately 9 months. These two parts can be described as follows:

I. Short-term Feeding Study—2 Months

Male and female weanling rats, weighing approximately 40-50 grams, were fed control diets containing 2 and 4 percent casein nitrogen and experimental diets with cottonseed flour supplying amounts of nitrogen equal to that in the two casein diets, as follows:

	Males	Females
Group I 2% Casein Nitrogen Control	10	10
Group II 2% Cottonseed Flour Nitrogen	10	10
Group III 4% Casein Nitrogen Control	10	10
Group IV 4% Cottonseed Flour Nitrogen	10	10
	40	40

The basal diet is described in Table I.

Table I

Basal Diet

Ingredients	Amount g	Vitamin	Amount mg/2400 g agar diet
Casein	250	Thiamine	4.8
Sucrose	64	Riboflavin	7.7
Dextrose	202	Nicotinic acid	96.0
Dextrin	159	Calcium pantothenate	96.0
Lard	232	Para-amino benzoic acid	96.0
Salt mixture	40	Pyridoxine	3.9
Agar	33	Choline	2400.0
Cod liver oil	10	2 methyl-naphthoquinone	.5
Alpha tocopherol (1%) in fat	10	Biotin	.5
Distilled water	1400	Folic Acid	.5
	2400	Inositol	240.0

[1] These studies were supported by a National Academy of Sciences grant RF-NRC-11 and represent part of a report sent to the sponsor.

Food intakes and body weights were recorded so that efficiency of protein utilization could be calculated. Hematological studies were made at the end of 8 weeks. At this time autopsies were done on five animals from each group, recording weights of liver, kidneys, heart and gonads. Specimens of these tissues were taken for histopathological examinations together with adrenals, spleen, lung, lymph nodes, thyroid, bladder, and portions of the gastrointestinal tract.

These experiments were expanded to compare the growth of rats when the nitrogen intake was altered, the nitrogen coming from different dietary protein sources. The protein concentration was varied from 0 to 50 percent by isocaloric interchange of protein for carbohydrate. Egg albumin, Labco casein, cottonseed meal, cottonseed meal plus 2.2 gm. lysine per 100 gm. protein, and wheat gluten were the protein sources studied.

Results of Short-term Feeding Study

The data in Table II demonstrate the lower rate of growth of the animals fed the cottonseed flour than those fed the casein. The protein-efficiencies (Grams gained in weight per gram of nitrogen intake) (see Table III), were much lower for the cottonseed flour than for casein when the diet contained 2% nitrogen. When the dietary nitrogen was increased to 4%, the differences between the efficiencies for casein and cottonseed flour were reduced.

Table II
Average Body Weights at the End of 4 and 8 Weeks of Feeding Casein or Cottonseed Flour

Source of Protein	Diet Nitrogen %	Body Weight 4 weeks	Body Weight 8 weeks
	Male		
Casein	2	157	236
Cottonseed	2	96	154
Casein	4	202	312
Cottonseed	4	148	221
	Female		
Casein	2	142	194
Cottonseed	2	106	153
Casein	4	143	195
Cottonseed	4	130	172

Table III
Protein Efficiencies (Grams Gained in Body Weight Per Gram of Nitrogen Intake) in Rats Fed Casein or Cottonseed Flour as Source of Nitrogen

Source of protein	Diet Nitrogen %	Nitrogen Intake grams 4 weeks	Nitrogen Intake grams 8 weeks	Protein Efficiencies 4 weeks	Protein Efficiencies 8 weeks
		Male			
Casein	2	4.8	10.8	22.7	17.5
Cottonseed	2	4.1	9.3	11.5	11.2
Casein	4	10.4	24.0	14.8	10.9
Cottonseed	4	9.5	20.7	10.4	8.3
		Female			
Casein	2	4.4	10.0	20.7	14.4
Cottonseed	2	4.3	9.4	12.6	10.7
Casein	4	9.7	20.0	9.4	7.2
Cottonseed	4	8.8	18.6	9.1	6.6

The blood cytology (Table IV) and organ weights (Table V) do not indicate any deleterious effects associated with the feeding of the cottonseed flour. Similarly, histopathological examinations of the various tissues did not reveal any abnormalities associated with the feeding of the flour.

Table IV
Blood Cytology at End of 2 Months of Feeding The Diets

Protein	Diet Nitrogen %	R. B. C. 106	W. B. C. 103	Hemoglobin g%
	Male			
Casein	2	8.21	9,800	14
Cottonseed	2	9.09	11,060	13
Casein	4	8.90	8,860	13
Cottonseed	4	10.15	7,920	14
	Female			
Casein	2	8.41	9,280	13
Cottonseed	2	8.90	6,830	13
Casein	4	9.43	12,960	14
Cottonseed	4	9.22	9,760	14

The experiments designed to compare cottonseed meal with other protein sources are summarized as follows. The increase in body weight with respect to nitrogen intake over a period of 28 days is called the nitrogen growth index (2).[2] These indexes were egg, 32; casein, 25; cottonseed flour, 15; cottonseed flour plus lysine, 18; wheat gluten, 6. The male rats approached 125 grams as the upper limit for gain in body weight during the period of

[2] Figures in parenthesis refer to References at end of this article.

Table V
Body and Organ Weights at End of 2 Months of Feeding The Diets

Source of Protein	Nitrogen %	Liver	Kidney	Heart	Gonad
		g/10c g. body weight			
		Male			
Casein	2	3.1 ± 0.18	0.71 ± 0.04	0.31 ± 0.01	1.1 ± 0.06
Cottonseed	2	3.3 ± .02	.65 ± .04	.31 ± .02	1.5 ± .05
Casein	4	2.8 ± .14	.66 ± .02	.27 ± .01	.88 ± .02
Cottonseed	4	3.1 ± .10	.68 ± .01	.31 ± .01	1.23 ± .06
		Female			
Casein	2	4.1 ± 0.11	0.79 ± 0.04	0.34 ± 0.01	0.07 ± 0.006
Cottonseed	2	3.5 ± .16	.76 ± .03	.37 ± .02	.06 ± .007
Casein	4	4.0 ± .25	.84 ± .02	.31 ± .01	.06 ± .007
Cottonseed	4	3.97 ± .14	.81 ± .004	.34 ± .02	.07 ± .006

28 days while the upper limit for females was approximately 100 grams. Maximum gain in weight in 4 weeks could not be reached by feeding proteins of low nutritive value such as wheat gluten even at the highest nitrogen intake.

II. Long-term Study on Reproduction and Lactation—Approximately 9 Months

A diet containing 4% casein nitrogen (see Table I) and one with an equivalent amount (in terms of nitrogen) of cottonseed flour was fed to male and female rats from weanling through the reproductive period, as follows:

		Males	Females
Group I	4% Casein Nitrogen Control	5	10
Group II	4% Cottonseed Flour Nitrogen	5	10

After physical maturity had been reached (roughly 2 months), Group I males were mated with Group I females, and similarly for Group II. The course of two pregnancies were followed with observations on fertility, course of pregnancy, and condition of the young.

The data obtained on reproduction are recorded in Table VI. The observations on fertility, course of pregnancy and condition of the young at birth indicate that rats fed cottonseed flour at the 4 percent nitrogen level reproduced normally. The offspring, however, grew at a slower rate when they were fed the cottonseed flour than when fed casein. These differences between body weights became insignificant, however, as the animals matured. For example, the body weight, together with the organ weights of the male and female parents at 10 months of age are recorded in Table VII. There are no significant differences at this age between animals fed casein or cottonseed flour (4% nitrogen levels). The

Table VI
Mating Experiment

Diet	No. of Conceptions	Ave. No. in litter	Ave. Wt. g.	No. of animals surviving for four weeks	Four Weeks No. Male	Four Weeks No. Female	Four Week Ave. Weight Male g.	Four Week Ave. Weight Female g.
			First Mating (Parents 3 months of age)					
Casein	3 out of 5	6.3	6.2	13 out of 19	11	2	111	97
Cottonseed	4 out of 8	7.0	6.9	26 out of 28	11	15	71	64
			Second Mating (Parents 7 months of age)					
Casein	3 out of 5	6.0	8.4	10 out of 18	4	6	110	100
Cottonseed	6 out of 8	6.8	7.2	36 out of 47	17	19	74	63

Table VII
Organ Wts. of Parents and of Offspring

Diet 4% Nitrogen	Body Wt. gm	Heart	Liver	Kidney	Gonad
			g per 100 g Body Weight		
Male Parents (10 Months)					
Casein	410 ± 23	0.305 ± 0.009	3.90 ± 0.34	0.856 ± 0.021	0.784 ± 0.033
Cottonseed	414 ± 10	.326 ± .028	3.62 ± .13	.679 ± .030	.700 ± .084
Female Parents (10 Months)					
Casein	320 ± 33	0.287 ± 0.028	3.69 ± 0.29	0.713 ± 0.019	0.0539 ± 0.0093
Cottonseed	263 ± 17	.350 ± .011	3.74 ± .22	.737 ± .009	.0869 ± .0260
Male First Mating (5 Months After Birth)					
Casein	344 ± 34	0.313 ± 0.008	3.70 ± 0.14	0.829 ± 0.041	0.840 ± 0.047
Cottonseed	327 ± 9	.333 ± .020	2.84 ± .17	.748 ± .020	.888 ± .081
Female First Mating (5 Months After Birth)					
Casein	282 ± 6	0.319 ± 0.012	2.99 ± 0.06	0.761 ± 0.003	0.0540 ± 0.0080
Cottonseed	219 ± 8	.343 ± .009	4.08 ± .25	.828 ± .029	.0674 ± .0080
Male Second Mating (2 Months After Birth)					
Casein	188 ± 25	0.449 ± 0.045	5.07 ± 0.28	1.19 ± 0.102	1.178 ± 0.023
Cottonseed	139 ± 13	.413 ± .019	6.13 ± .74	1.141 ± .051	1.398 ± .057
Female Second Mating (2 Months After Birth)					
Casein	163 ± 13	0.412 ± 0.024	4.48 ± 0.16	1.100 ± 0.055	0.519 ± 0.0086
Cottonseed	98 ± 14	.462 ± .038	5.32 ± .40	1.309 ± .213	.717 ± .0122

data obtained from offspring (first mating) at the end of 5 months are also recorded in this table. Similarly, data are recorded for offspring (second mating) at the end of 2 months. The smaller body weights of these younger animals fed cottonseed flour than those fed casein illustrate again the slower rate of growth of rats fed the cottonseed flour.

Samples of tissues were taken for histopathological examinations at the time of autopsy of all groups recorded in Table VII. These tissues were liver, kidney, heart, gonads, adrenals, spleen, lung, lymph nodes, thyroid, bladder, and portions of the gastrointestinal tract. No pathology was observed in any of these tissues that could be associated with the feeding of cottonseed flour.

III. Nitrogen Balance Indexes in Adult Dogs

A third study of the nutritive value of cottonseed flour involved the determination of nitrogen balance indexes in adult dogs. This index is a function of the amount of dietary nitrogen retained in the animal for protein anabolism and has the same significance as the "Biological Value." The index (K) can be calculated from the following equation:

$$\text{Index (K)} = \frac{\text{B-Bo}}{\text{A}}$$

Where B is the nitrogen balance produced while feeding the dietary nitrogen, Bo is the balance produced by feeding a protein-free diet, and A is the amount of dietary nitrogen absorbed into the animal. The amount of absorbed nitrogen (A) can be calculated from the total nitrogen intake (I) as follows:

$$\text{A} = \text{I} - (\text{F-Fo})$$

Where F is the fecal nitrogen excretion during nitrogen feeding while Fo is the excretion during a protein-free feeding period. Digestibility D, then is

$$\text{D} = \frac{\text{A}}{\text{I}}$$

and the nitrogen balance during the feeding of the protein-free diet Bo can be calculated as follows:

$$\text{Bo} = \text{Uo} + \text{Fo}$$

where Uo and Fo represent the excretion of urinary and fecal nitrogens respectively.

The data in Table VIII demonstrate that cottonseed flour is well digested in the adult dog and that the nitrogen balance index is 60 for unsupplemented flour. Past experience has indicated that an index of 60 or better will support normal growth in animals provided the nitrogen intake is sufficient for that purpose. The data presented for growth of rats prove that this cottonseed flour does support adequate growth in rats fed a sufficient nitrogen intake. Cottonseed flour is deficient in both methionine and lysine. Supplementation with either methionine or lysine improved the nutritive value in the adult dog as measured by the magnitude of the index. Supplementing with both methionine and lysine improved the index still further, making the supplemented protein of high nutritive value for maintenance in the adult.

Table VIII

The Determination of Digestibility and the Nitrogen Balance Index of Cottonseed Flour in Adult Dogs

Nitrogen Intake I	Urinary Nitrogen U	Urinary Nitrogen U	Urinary Nitrogen Uo	Fecal Nitrogen F	Fecal Nitrogen Fo	Nitrogen Balance B	Digestion D %	Index K
0.239 ± 0.007	0.210 ± 0.011		0.123 ± 0.007	0.060 ± 0.004	0.043 ± 0.004	—0.035 ± 0.012	93 ± 2	0.60 ± 0.04
			+ Methionine (1.2 g/100 g protein)					
.239 ± .006	.145 ± .004		.100 ± 004	.063 ± .002	.045 ± .003	+ .030 ± .007	93 ± 1	.79 ± .02
			Lysine (2.2 g/100 g protein)					
.247 ± .006	.152 ± .006		.089 ± .004	.057 ± .002	.035 ± .003	+ .038 ± .009	92 ± 1	.72 ± .02
			+ Methionine (1.2 g/100g protein) + Lysine (2.2 g/100g protein)					
.244 ± .007	.126 ± .006		.086 ± .004	.052 ± .002	.037 ± .003	+ .065 ± .010	93 ± 2	.85 ± .01

REFERENCES

(1) Allison, J. B., R. W. Wannemacher, Jr., R. Hilf, J. F. Migliarese, and M. L. Crossley, 1954. Dietary protein and tumor-host relationship in the rat. J. Nutrition, 54:593-600.

(2) Allison, J. B., R. W. Wannemacher, Jr., E. Middleton, and T. Spoerlein, 1959. Dietary Protein Requirements and Problems in Supplementation. Food Tech. 13: 597-602.

DISCUSSION

Harper: You referred to cottonseed and cottonseed flour in this work but you did not define the cottonseed meal or cottonseed flour you were using. Do you know anything of the characteristics of the samples which you were using?

Allison: They were given to me by UNICEF.

Allen: As far as I know, all of the flours that Dr. Allison has used in this work have been from the Traders Oil Mill; some of them were shipped directly from Traders Oil Mill and others were picked in the New York Warehouse. The data will be, I assume, quite similar to all the published data from Traders Oil Mill on their so-called Proflo products.

Question: What was the highest percentage of cottonseed flour fed in the diet to obtain optimum growth?

Allison: I'm not sure I can remember the exact percentage, but it would be around 30%.

Milner: I was interested in the rate of digestion or availability of amino acids of cottonseed flour being different from casein. Have you tried this with other seed proteins?

Allison: You understand that this difference in digestion is suggested as a working hypothesis. We've used it to explain some of our data, but we haven't tried other seed proteins. I would guess that eggs

would liberate into the intestinal tract almost an ideal pattern for absorption into the body for protein synthesis, but the pattern might differ from the chemical analysis for eggs. I may be going out on the limb to make a statement like this, but I want to suggest this possibility. In most studies, however, on supplementation with amino acids we get results that can be predicted from the chemical analysis.

Fincher: What is the effect of heat on the liberation of amino acids from cottonseed?

Allison: Heating can make amino acids more or less available but dry heat very often ties up lysine so that there is a reduction in the availability of lysine. In the case of soybean, probably heating makes methionine more available. In general I would say that cooking particularly in the presence of water, is an aid to digestion and the liberation of amino acids. Dry heat or certain types of overheating, whether in the presence of water or not, may tie up certain amino acids or destroy them so that the pattern of liberated amino acids is actually not improved but made worse.

I think that one of the greatest contributions that food technologists can make to the field of nutrition is to work out methods of preparing these foods so that the digestibility and also the pattern of amino acids presented to the body of the individual are improved. Therefore, I think that food technologists and nutritionists must work together to improve foods. I want to emphasize the importance of balance between various constituents of our diet as well as the balance between amino acids and the need to determine the best possible way to present a balanced diet to the different peoples of the world. We could use the argument in this country that we have so many different types of food, that we could educate housewives and ourselves to select a balanced diet. The food technologists therefore ought to help us present these foods in such a way that the housewife can know how to make and select the balance. To me, the most important feature we have in nutrition, is observe this optimum balance and then to present the foodstuff to the people in the world so that they get a balanced diet.

Ward: I thought perhaps Dr. Carl Lyman might have something to say along that question.

Lyman: It seems to me that the first thing that we recognize is the tremendous variation that we have in different samples of cottonseed flour. This variation is clear outside of the range that we would expect. In other words, the nutritive value of one sample of cottonseed meal or flour may be three times the other. Because of this, we need to know something about what kind of a sample we are testing if we compare with other materials such as casein or soybean flour, for example. The other thing to recognize is that a typical cottonseed flour may only have as much as 65% or so of lysine which is available —digestible at all—and this ranges all the way to 100% in arginine. So that not only is there a difference in rate of liberation of amino acids, but in some samples of cottonseed flour or meal, a proportion of the amino acids and particularly lysine is not liberated at all. Our calculations of what we have are more complicated because we must consider here the very important factor of amino acid availability.

THE USE OF COTTONSEED FLOUR IN VEGETABLE PROTEIN MIXTURES FOR HUMAN FEEDING
I. BIOLOGICAL STUDIES [1]

by

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The numerous surveys carried out in many of the Latin American countries have shown that corn consumed in many different forms is the most important staple food for the rural

[1] INCAP publication 1-184.

populations of the area. Table I shows the average consumption of corn in several countries in Central America and the average amount of protein and of calories contributed by the cereal grain to the rural diet. It is evident from the figures that corn provides significant amounts of the two nutrients to the daily rural diet. It is also well known that the nutritive value of the proteins of corn is extremely poor so that the actual amount of protein available for growth and maintenance is much less than that indicated by the total amount ingested. It is therefore of great practical importance to enrich corn diets so that they will provide better quality and a larger quantity of protein.

Table I
Daily Quantities of Corn Consumed in Rural Areas in Central America Per Person [1]

	Weight g.	Calories	% of Total Calories	Protein g.	% of Total Protein
Costa Rica	185	635	34	15	32
Nicaragua	300	1030	57	24	40
Honduras	398	1370	69	32	48
El Salvador	374	1286	65	30	58
Guatemala	423	1456	64	34	49

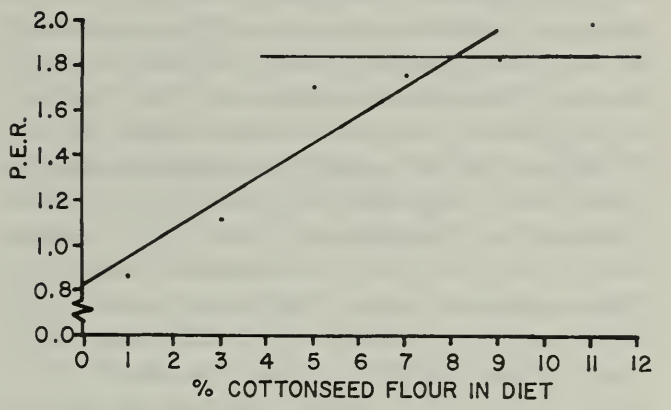
[1] Data Kindly Supplied by Miss Marina Flores, INCAP.

The protein enrichment of corn with cottonseed flour has been approached in two ways. One has been to find the minimum amount of cottonseed flour needed to bring about a maximum improvement in the nutritive value of corn proteins. The second approach, which is one of wider applicability in human nutrition, has been the enrichmant of corn and other cereals with cottonseed flour in the form of vegetables mixtures.

An example of the use of cottonseed flour for the enrichment of lime-treated corn flour is shown in Figure 1. This experiment was carried out with young rats. To a 79% lime-treated corn flour-basal diet, levels of 1 to 11% cottonseed flour were added. The figure shows the PER obtained at each level of cottonseed flour supplement. It is evident that maximum improvement was obtained with about 8% cottonseed flour in the diet.

In Table II the improvement in nutritive value of lime-treated corn with cottonseed flour is compared to the improvement obtained by adding other protein rich foods of both

SUPPLEMENTATION OF LIME-TREATED CORN WITH COTTONSEED FLOUR



animal and vegetable origin. In general, all protein rich foods used increased the nutritive value of the proteins of lime-treated corn. A higher improvement with smaller additions is evident in the animal protein group of supplements, although the vegetable protein supplements, including cottonseed flour, brought about a significant increase in the nutritive value of lime-treated corn proteins as judged by the PER.

Table II
Amount of Protein Rich Foods, Found Optimum For Supplementing Lime-Treated Corn

Protein Rich Foods	Amount Found % of Diet	Protein Efficiency Ratio
None	------	1.00
Egg Protein	3.0	2.25
Casein (V.F.)	4.0	2.21
Meat Flour	4.0	2.34
Fish Flour	2.5	2.44
Soy-bean protein	5.0	2.30
Soy-bean meal	8.0	2.25
Cottonseed flour	8.0	1.83
Torula yeast	2.5	1.97
Pumpkin seed flour	5.5	1.73

As mentioned previously, another approach to the problem of the improvement in the nutritive value of lime-treated corn has been through the development of protein rich vegetable mixtures (1,5,6).[2] It is now widely accepted that an important and practical approach to supplying the needed dietary protein

[2] Figures in parentheses refer to References at end of this article.

in areas where milk and other products of animal origin are costly or in short supply is the development of suitable combinations of vegetable protein sources to supply both essential and non-essential amino acids in the quantity and proportions required. The first of the vegetable protein mixtures developed by INCAP to receive extensive biological testing is known as Vegetable Mixture No. 8 (1,2,3). It consists of lime-treated corn 50% sesame flour (33% fat) 35%, cottonseed flour 9%, Torula yeast 3% and Kikuyu leaf meal 3%. This mixture contains approximately 25% crude protein with a protein score of 67% based on the FAO amino acid reference pattern (4).

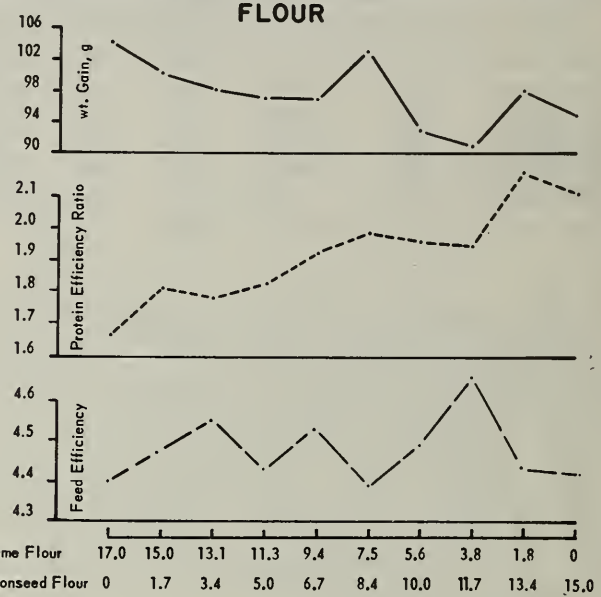
Although in chicks, rats, and children (1,2, 3,9) Vegetable Mixture No. 8 proved to be good protein source, it was not sufficiently economical for the Central American area because of the short supply of sesame seed. A less expensive mixture eliminating sesame flour was developed using cottonseed flour as the vegetable protein concentrate. The relative nutritive quality of cottonseed flour as a substitute for sesame flour was first studied as part of the development of INCAP Vegetable Mixture 9. The results of a representative chick growth experiment are shown in Table III. Cottonseed flour was added to replace sesame flour isoproteically. It can be seen that the growth of the chicks and the feed efficiencies after 35 days on trial improved as cottonseed flour replaced sesame flour. From this and other experiments (7,8), it was concluded that the nutritive value of cottonseed flour protein for this purpose is higher than that of sesame flour, presumably due to a higher lysine content in cottonseed flour. Results of a similar experiment carried out with young growing rats are shown in Figure 2. The gain in weight and feed efficiencies were similar in all groups as cottonseed flour protein replaced sesame flour. Protein efficiencies, however, improved from 1.62 when the corn based diet contained sesame flour to 2.10 when it contained only cottonseed flour. Again it was evident that properly processed cottonseed flour is a good protein supplement for corn proteins and somewhat superior to sesame flour.

Table III
Substitution of Sesame Flour by Cottonseed Flour [1] [2]

Percentage in Diet		Final Weight	Feed
Sesame Flour	Cottonseed Flour	g [3]	Efficiency [4]
34.00	0	235	2.97
30.00	3.43	225	3.08
26.25	6.75	253	2.86
22.50	10.07	260	2.84
18.75	13.39	283	2.86
15.00	16.71	244	3.46
11.25	20.03	312	2.86
7.50	23.35	336	2.71
3.75	26.67	342	2.58
0	30.00	337	2.69

[1] No. of Chicks/group, 12.
[2] Percentage protein in Diet, 20.4
[3] Experimental period 35 days.
[4] Average feed consumed/Average weight gained.

SUBSTITUTION OF SESAME FLOUR BY COTTONSEED FLOUR



In order to determine the optimum protein combination between corn and cottonseed flour protein, experiments were carried out in chicks and young rats in which the protein of the diets was contributed by various combinations of the two foods. The results of a representative chick trial are shown in Table IV. Gain in weight and feed efficiencies were superior when 15-25% of the protein of the diet was from corn and 85-75% from cottonseed flour. In Figure 3, the results in young rats are described. The bars in the lower part of the figure represent the dietary protein distribution. The curves from top to bottom are gain in weight, feed and protein efficiencies, re-

Table IV
Optimum Protein Combination Between Corn and Cottonseed Flour in Chicks

10 Chicks/group

% Protin Distribution in Diet [1]		Weight Gained g. 28 days	Feed Efficiency [2]
Corn	Cottonseed Flour		
10.3	89.7	228	2.19
15.3	84.7	194	2.08
20.3	79.7	284	1.92
25.4	74.6	307	2.00

[1] Percentage protein in the Diet 22.5.
[2] Average Feed Consumed/Average Weight Gained.

spectively. It can be noticed that better gains in weight and higher protein efficiencies are obtained when corn and cottonseed flour provide 15 and 85% of the protein of the diet. It is also apparent that when cottonseed flour contributes less than 70% of the protein of the diet, gain in weight, protein efficiency and feed efficiency decrease (7).

The essential amino acid pattern of the 15 to 85% protein corn-cottonseed flour mixture is shown in Table V. The supplementary value of cottonseed flour in the proportions found experimentally are evident. Comparison with the FAO pattern (4) reveals deficiencies in isoleucine, lysine, total sulphur, amino acids and tryptophane. Addition of cottonseed flour to corn improves but does not wholly correct the lysine and tryptophane deficiency in the cereal grain according to the FAO pattern. The amino acid proportions in the 15 and 85% protein mixture, using tryptophane as the base, are similar to those of the FAO pattern.

From experimental results of this type and corn and cottonseed flour, INCAP Vegetable Mixture 9 was formulated. The corn-cottonseed protein combination chosen for Vegetable Mixture 9 was the one in which corn provides 20% of the protein and cottonseed flour 80%. This meant that the maximum protein content in the final mixture would be from 26 to 28%. Vegetable Mixture 9 was then formulated as follows in percent: 56 whole ground corn, 38 cottonseed flour, 3 Torula yeast and 3 dehydrated leaf meal. Experimental results also indicated that sorghum grain could replace all or part of the corn (3,8), and to lower its cost, the experimental formula was made of 28% whole ground corn, 28% ground sorghum grain, 38% cottonseed flour, 3% Torula yeast and 3% dehydrated leaf meal.

This formula was then subjected to extensive testing in chicks, rats, dogs, and pigs (10,11,12). A representative chick trial is shown in Table VI. Chick growth and feed efficiencies are similar when the mixture is made with 28% corn and 28% sorghum, with 56% corn or with 56% sorghum. The response is 82% of that obtained with a commercial chick feed containing animal protein. As shown in the lower part of Table VI, supplementations with lysine significantly improved

COMBINATIONS BETWEEN COTTONSEED FLOUR AND CORN PROTEINS

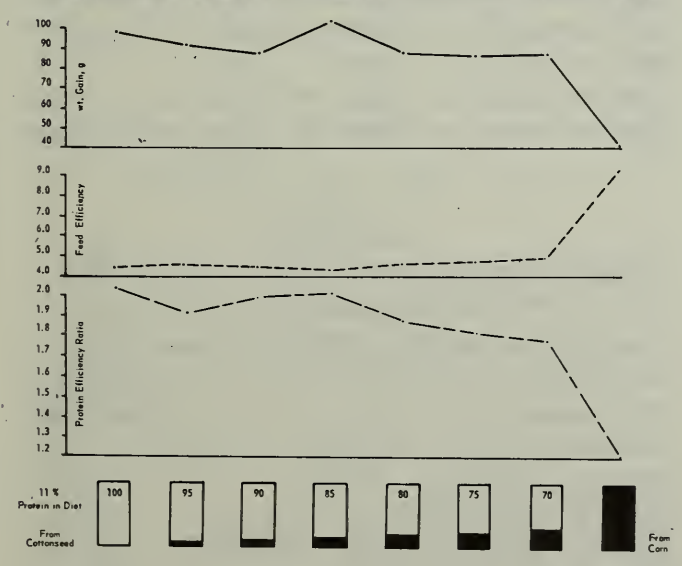


Table V
Essential Amino Acid Patterns

Amino Acid	Corn	Cottonseed Flour	15-85 Protein Combination Corn-Cottonseed	FAO Ref. Prot.
		mg/g N		
Arginine	262	719	647	------
Histidine	231	113	132	------
Isoleucine	213 [1]	231 [1]	228 [1]	270
Leucine	572	413	437	306
Lysine	126 [1]	256 [1]	235 [1]	270
Methionine Cystine	189 [1]	169 [1]	172 [1]	270
Phenylalanine	276	294	291	180
Threonine	214	294	281	180
Tryptophan	32 [1]	75 [1]	68 [1]	90
Valine	281	331	323	270

[1] Limiting Amino Acids.

growth of the chicks fed Vegetable Mixture 9 and methionine addition had a small effect of doubtful significance.

Table VI
Representative Chick Growth Trial With INCAP Vegetable Mixture 9′

(35 DAYS—20 CHICKS PER GROUP)

Diet	Protein in Diet %	Final Weight g.	Feed Efficiency
V.M.9′	23.5	479 [1]	2.31
V.M.9′ with 56% Corn	23.8	460 [1]	2.25
V.M.9′ with 56% Sorghum	24.1	479 [1]	2.27
"Ace-Hi"	23.9	587 [1]	2.01
V.M.9′	23.0	310 [2]	2.45
" + 0.3% DL-Met	23.0	361 [2]	2.26
" + 0.2% L-Lys HC1	23.0	472 [2]	2.14
" + both A.A.	23.0	490 [2]	2.04

[1] 55 g. initial weight. [2] 45 g. initial weight

Since other cereal grains are more important staple foods in other regions of the world than corn and sorghum, the results of a chick experiment fed Vegetable Mixture 9 in which the corn-sorghum combination was substituted for several other cereal grains, are shown in Table VII. In general, both the growth of the chicks and the feed efficiencies in 35 days were very satisfactory with these other cereals and rice gave the best results. It can be concluded that other cereals can be used in the formula of Vegetable Mixture 9 without lowering the nutritive value of the mixture.

A representative rat growth experiment is shown in Table VIII where Vegetable Mixture 9 was fed at five levels of protein in the diet and the response compared with that obtained by feeding five levels of protein from casein.

Table VII
Substitution of Yellow Ground Corn In Vegetable Mixture 9 By Other Cereal Grains [1]

Cereal Grain in V.M.9′	% Protein in Diet	Final Weight [2] g.	Feed Efficiency [3]
Yellow Corn	24.5	399	2.17
Wheat Flour	25.3	357	2.32
Barley	25.9	420	2.27
White Rice	24.7	426	2.07
Oats	24.7	383	2.30
Whole Wheat	25.8	380	2.41

[1] Chicks per group, 17.
[2] Experimental period, 35 days.
[3] Average feed consumed/Average weight gained.

Table VIII
Representative Rat Growth Experiment

(21 DAYS—6 RATS/GROUP)

Protein in Diet %	Average Weight Gain g. V.M.9	Average Weight Gain g. Casein	Protein Efficiency [1] V.M.9	Protein Efficiency [1] Casein
5	22	22	1.88	2.14
10	66	67	2.30	2.38
15	104	105	2.11	2.31
20	114	117	1.75	2.00
25	115	121	1.47	1.65

[1] Ave. Wt. Gain/Ave. Protein Consumed.

Weight gains in 21 days were similar for both proteins, however, the protein efficiency ration of casein was higher than the PER of the vegetable mixture, particularly at low protein levels in the diet. A representative rat protein-repletion study is shown in Table IX. Good repletion weights were observed in the rats fed Vegetable Mixture 9 at the 10% protein level in the diet and whether the mixture was made with 56% corn, 56% sorghum or 28% corn plus 28% sorghum grain. The response from all diets was similar to that obtained with skim milk fed at iso-proteic levels. The results presented in Table X show the effect of supplementing Vegetable Mixture 9 at the 10% level of protein in the diet, with lysine and methionine added alone and measured in the protein-depleted rat. It is evident that addition of 0.20% L-lysine HC1 improved repletion weight gains while higher lysine levels did not. Methionine addition at any level tested did not improve the nutritive quality of Vegetable Mixture 9.

Representative tests with young growing dogs are given in Table XI. This table shows

Table IX
Representative Rat Repletion Trial With INCAP Vegetable Mixture 9′

(10% PROTEIN IN DIET, 6 RATS PER GROUP)

Mixture	Ave. Wt. Gain in 15 days
	g.
V.M. 9′ (56% Corn)	63
V.M. 9′ (28% Corn, 28% Sorghum)	61
V.M. 9′ (56% Sorghum)	69
Skim milk	66

Table X
Representative Rat Repletion Study
Amino Acid Supplementation of
INCAP Vegetable Mixture 9
(10% PROTEIN IN DIET, 6 RATS PER GROUP)

A.A. Added	g%	Repletion Gain in 14 days
		g.
None	0.0	51
L-Lysine HC1	.1	60
L-Lysine HC1	.2	71
L-Lysine HC1	.3	62
DL-Methionine	.1	53
DL-Methionine	.2	51
DL-Methionine	.3	53

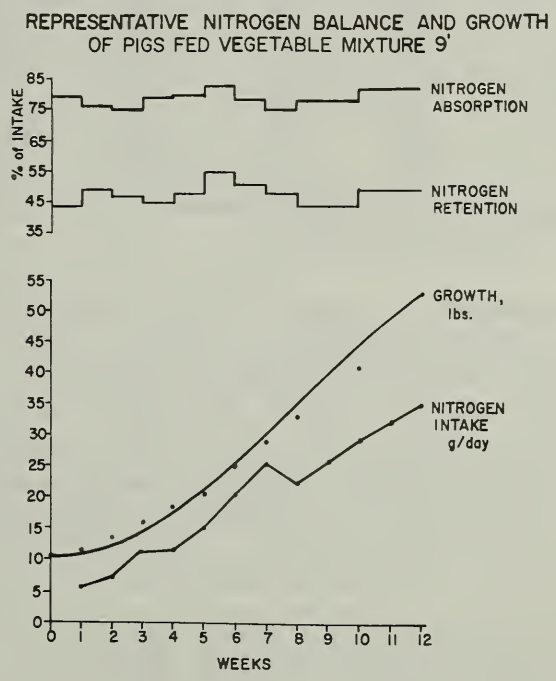
the biological value of Vegetable Mixture 9 as compared with casein. The study was carried out by feeding the dogs from 3.0 to 4.5 gm. of protein per K. of body weight per day of either casein or Vegetable Mixture 9. A nitrogen-free feeding period was also included to obtain data on endogenous urinary and metabolic fecal nitrogen to be applied in the formula of Thomas and Mitchell (13,14). The average biological value for casein was 78% while that for Vegetable Mixture 9 was 74%.

Table XI
Biological Value of Vegetable Mixture 9 and Casein

Dog No.	V.M.9' Protein Intake g/k/day	B.V. [1]	Casein Protein Intake g/k/day	B.V. [1]
7	3.52	76.3	3.81	78.1
8	3.29	71.0	3.92	73.4
9	4.04	76.0	4.02	78.4
11	4.48	70.7	4.43	82.1
Ave.	------	73.5	------	78.0

[1] $B.V. = \frac{NI-(FN-MN) - (UN - EN)}{NI-(FN-MN)} \times 100$

The mixture was tested further in young growing pigs. The results are shown in Figure 4. The pigs were fed Vegetable Mixture 9 cooked in water for half an hour in the proportions of one part solid to ten parts liquid. After cooking the vegetable mixture, minerals and vitamins were added to reduce moisture content and when cool it was fed to the pigs. The upper part of the figure shows the weekly percentage of nitrogen absorption and retention of nitrogen intake over a 12-week period. The lower part of the figure indicates the nitrogen intake in grams per day and the growth curve in pounds, respectively, over the 12-week period. Nitrogen absorption and retention were very consistent and high during the whole experimental period and the growth of the pigs was satisfactory. No signs of ferocity were noticed at any time.



One of the most important factors limiting the use of cottonseed meals as a food for humans has been the presence of gossypol pigments and their effect on the quality of cottonseed protein. Since Vegetable Mixture 9 contains 38% cottonseed flour and has a gossypol content of .016%, it was of interest to find what effect cooking had on the pigment. Representative results of two experiments are shown in Table XII. In both experiments, samples were analyzed for free and total gossypol after cooking for 5,10,15,20, and 25 minutes. In the second and third column the gossypol content is given on samples that were cooked in the laboratory with constant stirring, using 20 gm. of mixture and 200 ml. of water. After cooking, the material was lyophilyzed and analyzed. Columns 4 and 5 show the results of cooking Vegetable Mixture 9 using the same ration of solids to liquids as before by determining the gossypol in the wet material immediately after cooking and cooling. Moisture determinations were then carried out and the values presented are on a moisture-free basis.

Table XII
Changes in Free and Total Gossypol of Vegetable Mixture 9 During Cooking

Cooking Time Minutes	V.M.9' [1] Gossypol Free mg/100g	V.M.9' [1] Gossypol Total g%	V.M.9B [2] Gossypol Free mg/100g	V.M.9B [2] Gossypol Total g%
0 dry	15.8	0.45	15.8	0.42
0 wet	17.3	.46	6.8	.43
5	15.0	.49	7.4	.35
10	12.9	.47	6.4	.46
15	11.4	.45	8.7	.42
20	8.8	.44	5.1	.38
25	10.2	.44	3.7	.42

[1] Analysis on freeze-dryed cooked sample.
[2] Analysis on cooked wet sample.

The results in columns 2 and 4 show that free gossypol decreases as cooking time increases, the drop being more marked in the samples analyzed in the wet state. It was noticed repeatedly that a drop in free gossypol occurred immediately after wetting the sample. Total gossypol did not change in either group of samples during cooking. The drop in free gossypol could be explained as the binding of the pigment to epsilon amino-lysine groups in the protein of the material. Moreover, it is also known that in many vegetable proteins as much as 20% of the total nitrogen is nonprotein in nature. It is thus theoretically possible that some of the lysine binding occurs with free amino acids or other nonprotein nitrogenous constituents.

One of the most important requirements in the development and use of low cost vegetable protein mixtures for human feeding in technically underdeveloped areas is that they can be formulated from materials grown and processed in the area where they are to be used. Cottonseed cake samples from mills in several of the Central American countries were analyzed to find one suitable for use in the Mixture 9 formula. A cottonseed meal produced in El Salvador by prepress solvent extraction operations met WHO-FAO-UNICEF Protein Advisory Group specifications in all essential respects. The screened flour incorporated in the formula of Vegetable Mixture 9 as the sole source of protein was tested biologically in chicks. Its performance was compared to that obtained from "Pro-flo" cottonseed flour fed in the mixture and alone. The results of several such experiments are shown in Table XIII. It is evident that the "Borgonovo" cottonseed flour from El Salvador, as judged from weight gains and feed efficiencies after an experimental period of 28 days, is nutritionally superior to "Pro-flo" flour when fed either in the vegetable mixture or as the only source of protein. The chemical analysis of the two flours given in Table XIV shows them to be similar in composition although the "Borgonovo" flour is slightly higher in both lysine (g./16g. N) and total gossypol. In the lower part of the table PER and FE values obtained from rat studies with the two flours are shown; they again indicate a higher nutritive value for the Salvadorenean cottonseed flour.

Table XIII
Evaluation of Borgonovo Cottonseed Flour Alone and in Vegetable Mixture 9

Trial No.	Cottonseed Flour	No. of Chicks Initial	No. of Chicks Final	Average Wt. g. Initial	Average Wt. g. Final [3]	F.E. [4]
1	Borgonovo [1]	10	10	55	246	2.73
	Pro-flo [1]	10	10	55	212	3.16
2	Borgonovo [1]	10	9	46	277	2.09
	Pro-flo [1]	10	10	46	222	2.49
3	Borgonovo [1]	10	10	46	258	2.05
	Pro-flo [1]	10	10	46	200	2.85
1	Borgonovo [2]	10	10	52	301	2.30
	Pro-flo [2]	10	10	53	233	2.68
2	Borgonovo [2]	10	9	46	317	2.00
	Pro-flo [2]	10	9	46	237	2.20

[1] In Vegetable Mixture 9'
[2] Tested Alone
[3] Experimental period 28 days.
[4] Average feed consumed/ Average Wt. gained.

Table XIV
Comparison of Pro-flo and Borgonovo Cottonseed Flour

Measurement	Pro-flo	Borgonovo
	Percent	
Moisture	3.17	11.19
Total Nitrogen	8.21	8.00
Protein (N x 6.25)	51.34	50.00
Ether Extract	5.21	4.25
Ash	6.61	--------
Crude Fiber	3.68	3.63
Soluble Nitrogen	57.4	73.8
Lysine (g/lb g N)	3.8	4.5
Free Gossypol	0.045	0.044
Total Gossypol	0.92	1.15
P. E. R. [1]	1.82	2.14
F. E. [1]	4.65	4.19

[1] As determined in INCAP

SUMMARY

As a partial solution to the protein malnutrition problem existing in Latin America and many other parts of the world, INCAP initiated in 1951 work on the development of all-vegetable protein mixtures intended primarily for the supplementary feeding of young children. Mixture 8, the first formula to be extensively tested, contained lime-treated corn 50, sesame flour 35, cottonseed flour 9, Kikuyu leaf meal 3 and Torula yeast 3%. This formula was found to have a good protein quality in tests with chicks, rats and children, but the price of the sesame in Central America was too high for practical use.

Experiments were then carried out to determine the optimum protein combination between corn and cottonseed flour. Studies in chicks and rats indicated that cottonseed flour could replace sesame flour in Mixture 8. The best combinations were those in which corn provided 15-20% and cottonseed from 80-85% of the protein of the mixture. Because of the relative abundance and low cost of cottonseed oil meal in Central America, a formula containing a larger amount of cottonseed flour was developed in 1958. It contained cottonseed flour 38, corn 28, sorghum 28, dehydrated leaf meal 3 and Torula yeast 3%.

This formula, identified as Mixture 9, was subjected to extensive biological testing. In chicks, the mixture produced good growth and feed efficiencies and no toxic effects were detected. Other cereal grains can replace totally or partially the corn and sorghum, but Torula yeast contributes toward the protein quality of the mixture, whose first limiting amino acid is lysine. Experimental results with rats also indicated the mixture to be of a quality comparable to milk, casein and meat flour. When fed at moderate protein levels, a deficiency is apparent which can be corrected by addition of 0.1% lysine and 3% of protein concentrates rich in this amino acid, added in place of the leaf meal. Two generations of rats were maintained without any apparent toxic effects.

Dogs were fed the mixture from 4 weeks of age up to 4 months. Biological value determinations in dogs average 74% for Mixture 9 and 78% for casein. Pigs were also fed the mixture from 5 weeks to 4 months of age with no apparent toxic effects. Free and total gossypol determinations on the cooked mixture indicated a decrease in free gossypol with cooking.

It is concluded that the mixture is of good protein quality, free of toxic effects, and suitable for human feeding. In all experiments reported, "Pro-flo" cottonseed flour was used. Recent experiments with a cottonseed flour produced by Borgonovo Hnos., El Salvador, gave slightly better results.

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DISCUSSION

Question: Did you measure available lysine in the cottonseed flours that you used?

Bressani: We did not measure available lysine by determining the free epsilon amine nitrogen. We did measure available lysine *in vivo* in chicks and concluded that for the cottonseed protein samples that we have, about 80% of the total lysine (as measured microbiologically) is available.

Aines: How was the Borgonova meal produced?

Bressani: It was produced by prepress solvent extraction.

Question Is the Borgonova cottonseed flour being used in your present mixtures?

Bressani: Yes. Dr. Scrimshaw is going to talk about this.

Question: With what experimental animals have you tested the Borgonova flour?

Bressani: We have tested it with dogs, rats, and chicks.

Question: What is the capacity of the Borgonova plant?

Paggi: The capacity of the Borgonova plant in Salvador is about 80 tons per day.

THE USE OF COTTONSEED FLOUR IN VEGETABLE PROTEIN MIXTURES FOR HUMAN FEEDING II. CLINICAL TRIALS[1]

by

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An important part of the high mortality in most underdeveloped countries is due directly or indirectly to an inadequate consumption of a sufficient quantity and quality of protein in the diet. While much can be done to increase the availability of animal protein, its cost and

[1] INCAP publication I-185.

the pressure of growing populations makes the utilization of suitable vegetable sources of protein important. For tropical and sub-tropical regions cottonseed flour is one of the most promising sources of additional protein for human diets, providing it comes from high quality seed processed in such a way as to be relatively low in gossypol and with protein quality intact. Dr. Bressani has described the basic INCAP experimental work in animals that has led to the development of vegetable mixture formulas using such cottonseed flours for the supplementary and mixed feeding of infants and young children and for improving the protein value of the diets of persons of all ages. Other speakers at this meeting have discussed the specifications for cottonseed flour of suitable quality and described the agricultural and industrial problems involved in its production. This presentation will summarize INCAP clinical experience with the various vegetable mixtures containing cottonseed flour. These have not only been shown to be widely acceptable in the diets of persons without obvious malnutrition but also have been found effective in the treatment of severe protein malnutrition in children (kwashiorkor).

Material and Methods

Initial acceptability and metabolic trials were carried out in children 1 to 5 years of age under study in the INCAP metabolic unit and in an advanced stage of recovery from kwashiorkor. As experience was acquired the formulas were also used in the early and even initial treatment of children with kwashiorkor. The various formulas involved in this program are given in Table I.

Table I
Formulas for INCAP Vegetable Mixtures For Human Feeding

Ingredient	8	8A	9	9A	9B
Lime-treated	50	50	28	----	----
Corn Cooked	----	----	----	29	----
Uncooked	----	----	----	----	29
Sesame flour 33% fat	35	----	----	----	----
18 fat	----	35	----	----	----
Lime-treated	----	----	28	----	----
Sorghum Cooked	----	----	----	29	----
Uncooked	----	----	----	----	29
Cottonseed flour	9	9	38	38	38
Torula yeast	3	3	3	3	3
Dehydrated leaf meal	3	3	3	----	----
CaCO3	----	----	----	1	1
Vitamin A	----	----	----	4500	4500

Mixture 8A differed from 8 only in the use of a sesame seed flour of lower fat content. Mixture 9', the uncooked form of Mixture 9, was used only in the animal feeding experiments. From the standpoint of protein quality, there was no difference between mixtures 8 and 8A or between 9, 9A and 9B, although the latter two did not employ lime-treated corn and had the leaf meal as a source of vitamin A activity replaced by the synthetic vitamin.

Mixture 9A, with the corn and sorghum precooked, received 10 minutes of final cooking after blending one part of the dry powder into approximately 10 parts of water; 9B, utilizing raw corn and sorghum, required 15 minutes cooking under the same circumstances. All preparations were served primarily as cereal gruels with added sugar, and flavored with cinnamon, vanilla or chocolate.

The cottonseed flour employed in all of the experimental testing in children was "Pro-flo," produced by the Traders Oil Mill, Fort Worth, Texas. The large-scale commercial trial in Guatemala with INCAP Vegetable Mixture 9B used a cottonseed flour produced by Borgonovo Hermanos, El Salvador, whose characteristics have been described by Dr. Bressani (1).[2] Specifications and sources for other components have been previously described.

Mixture 8 contained approximately 25.1% protein and Mixture 9, 27.5%. As described in detail elsewhere (2,3,4), both mixtures contained a balanced complement of other essential nutrients except ascorbic acid. The chemical analysis and biological trials cited by Dr. Bressani (1, 5) indicated that, as a protein source, each should give results in children similar to those obtainable with skim milk.

Therapeutic and Metabolic Studies

After preliminary tests of palatability and initial tolerance, Mixture 8 with its content of 9% cottonseed flour was given as the sole source of protein to seven children who had recovered from the acute phase of kwashiorkor but who were not yet ready for discharge from the hospital (2,4,6). The longest periods for

[2] Figures in parenthesis refer to References at end of this article.

which Vegetable Mixture 8 was fed as the sole source were 59, 50 and 33 days. In every case the clinical response was excellent and the mixture resulted in firm and regular stools. Following this favorable experience, 6 children with full-blown kwashiorkor were treated from the time of admission with Mixture 8 as the sole protein source with a clinical response indistinguishable from that usually achieved with milk (2,4). The recovery of one of these children is illustrated by the first child in Figure 1.

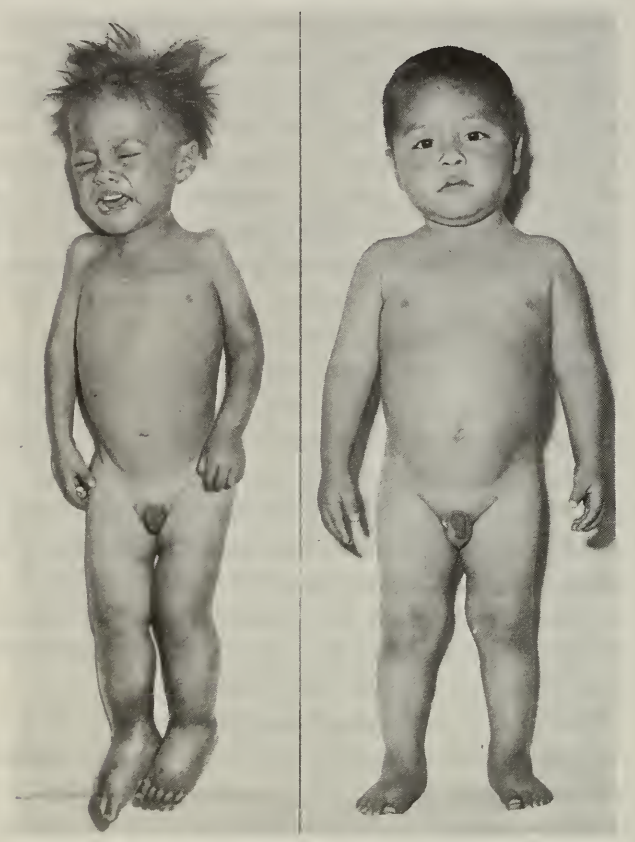


Fig. 1. Child PC-88 in the two photographs was a boy 2 years, 8 months, weighing 18 lbs., 7 oz. on admission to the hospital and treated with INCAPARINA as a sole source of protein. He is shown on the right after 4½ months of this treatment at which time he weighed 22 pounds.

In eight of these children, 5 day nitrogen balance periods with the vegetable mixture as the protein source were compared with similar periods when milk was given isonitrogenously. Table II, taken from a recent publication (4) shows that at levels of intake between 2.00 and 2.8 gm., there was no difference between the average retention of nitrogen expressed as percent of intake when the protein was derived from Vegetable Mixture 8 and that when it was supplied by cows milk. Mixture 8A gave equally satisfactory results when used in balance experiments but was less well tolerated for the initial therapy of kwashiorkor due to unknown differences in the sesame flour which were unrelated to the value of cottonseed flour in the formula.

Tolerance of patients to Mixture 9 was established during nitrogen balance studies in 3 children and treatment of 2 children partially recovered from kwashiorkor. Table III, from a recent INCAP publication (4), summarized the results reported to date from 72 five-day balance periods with one or another of the Mixture 9 formulas and a similar number of control periods in which milk was the protein source. At levels of protein intake of 2.0 gm. and above, the retentions obtained with the Vegetable Mixtures 9A and 9B were fully comparable to those obtained with milk, al-

Table II
Comparison of Vegetable Mixture 8 and Milk in Young Children

				MILK				VEGETABLE MIXTURE 8			
Children	Age	Weight	Cal/kg	Days	Intake g/kg	Absorption % of intake	Retention % of intake	Days	Intake g/kg	Absorption % of intake	Retention % of intake
48	1 yr. 9 mos.	7.5	101	5	2.5	87	0	5	2.3	73	11.3
23A	3 yrs.	11.2	106	5	2.6	74	23.2	5	2.6	78	23.2
48	1 yr. 9 mos.	9.9	110	5	3.8	90	14.8	5	3.8	71	12.0
56	3 yrs. 6 mos.	9.5	101	5	2.8	74	11.0	5	3.0	71	13.5
57	4 yrs. 3 mos.	11.6	108	5	2.9	80	33.6	5	2.8	68	22.5
73	1 yr. 1 mo.	7.4	100	5	3.0	83	17.6	10	3.5	78	31.3
75	1 yr. 3 mos.	7.5	95	10	2.6	83	38.2	5	2.9	70	23.8
88	3 yrs.	10.7	108	5	2.0	78	25.9	10	3.3	67	15.2
Average			104		2.8	81	20.5		3.0	72	19.1

though once again, the absorption, expressed as percent of intake, was slightly higher with milk. At levels below 2.0, inadequate for children of the age studied, the retentions with Vegetable Mixture 9 were still strongly positive although somewhat higher levels were attained with milk.

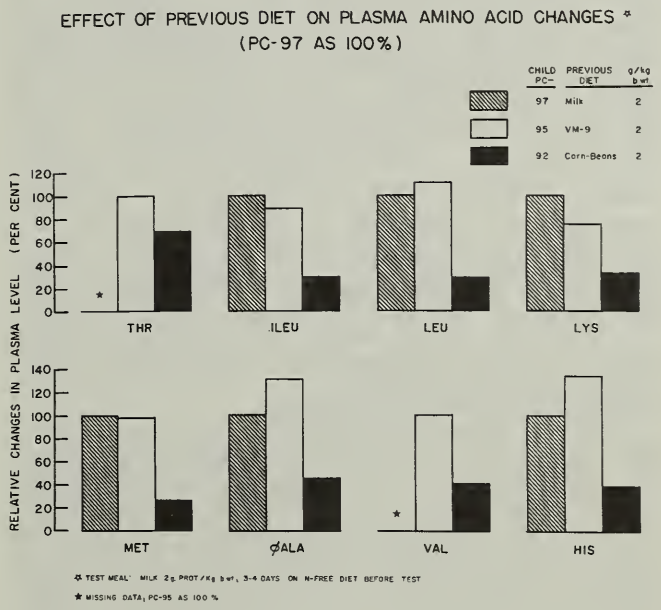
Five children with kwashiorkor admitted to the hospital in this period were treated with Vegetable Mixture 9 with results not distinguishable from those previously observed with milk and with Mixture 8. One of these is illustrated in Figure 1.

These results were highly gratifying but did not in themselves assure that any of these mixtures could be successfully introduced for the local use of persons with growing children and grossly inadequate economic resources. This could be determined only by field acceptability and marketing trials.

Special Biochemical Findings

A new approach for evaluating the quality of dietary protein in human subjects is under investigation in INCAP by Arroyave (7). It involves the determination of the plasma amino acid pattern by ion exchange column chromatography before and after a small test feeding of milk. Observations in dogs and children indicate that the plasma amino acids increases, after a standard protein feeding, are influenced by the nutritional state of the subject as determined by the previous diet. A decreased response seems to occur if a protein of poor nutritional value has been fed for some time before the test.

In two separate 4-week experiments each of three children was fed either a diet containing Vegetable Mixture 9B as the sole protein source, milk or a mixture of corn and beans in which each furnished half of the protein. After a fasting blood sample, a test meal of skim milk providing 2 gm. of protein/k. of body weight was administered and a second blood sample taken 2-½ hours later. The shift in plasma amino acid for the first of these trials is given in Figure 2, taken from a recent publication, and the results with the second were almost identical.



They show that the changes in the child fed the vegetable mixture were essentially the same as those of the child receiving milk and very much higher than those of the child who had been subsisting on the corn and beans diet. The results tend to confirm the impressions of the metabolic and therapeutic trials that the

Table III
Comparison of Vegetable Mixture 9A or 9B and milk in Young Children [1]

			MILK			VEGETABLE MIXTURE 9A Or 9B		
g prot/kg	No. Children	No. balance Periods	Intake g/kg	Absorption % of Intake	Retention % of Intake	Intake g/kg	Absorption % of Intake	Retention % of Intake
4.0	1	2	4.0	84.4	22.0	4	66.1	24.1
3.0-3.9	4	11	3.0	84.9	17.1	3.0	70.2	16.8
2.0-2.9	9	48	2.3	82.6	16.3	2.3	68.9	17.8
1.0-1.9	4	13	1.2	78.1	24.9	1.2	66.2	15.5
1	2	3	0.5	67.2	8.1	0.5	59.1	4.5

[1] Scrimshaw et al. (ref. 4)

quality of the protein in Vegetable Mixture 9 is indistinguishable by these means from that of milk, at least at normal and therapeutic levels of protein intake.

Field Studies

Field acceptability trials were carried out only with formulas 9A and 9B used interchangeably in a total of 129 children in four Guatemalan communities for periods of 15 to 19 weeks (4, 8). The mixtures were supplied to the mother in plastic bags containing 75 gm., each sufficient for the preparation of the three daily glasses of INCAPARINA which were recommended. An average of 78% of all children in the study consumed 2 or more glasses daily. Ninety-seven percent of the glasses offered were accepted, and only 3% rejected; no instance of intolerance to the mixture was encountered. Subsequently, similar results were obtained in El Salvador where 53 children were given the mixture for 4 weeks, and in preliminary acceptability trials in Honduras and Nicaragua.

These studies established the acceptability of the product when offered without cost but did not measure the willingness of the parents to purchase the preparation for their child or for the entire family. Arrangements were then made by INCAP and the government of Guatemala for a market trial in the predominantly Indian village of Palin with an estimated population of 4,000. The mixture was marketed under the generic name of INCAPARINA using the same type of plastic bag containing 75 gm. of powder which had been employed in the acceptability trials. Within 3 weeks sales stabilized at approximately 1,200 bags per week and remained at this average level for the 5-month trial period. No commercial advertising was used, but the product was recommended by personnel of the health center and by the local school teachers.

The name INCAPARINA has now been adapted as a generic name to refer to any vegetable mixture developed by INCAP suitable for feeding to young children and containing at least 25% protein of a quality comparable to that of milk and other products of animal origin.

Similar arrangements were made for larger place marketing trials to involve 43 communities with health centers or units and limited commercial advertising. This trial began March 22, 1960, and was terminated September 30, 1960, at which time over 840,000 of 75 gm. portions had been sold. Initial demand for the product was unexpectedly high and the full production was absorbed by the two principal cities and nearby towns. For this reason, the original plan could not be carried out because adequate production and packaging facilities were lacking. For the same reason, almost no commercial advertising was employed. The response was so encouraging, however, that negotiations are in progress for large-scale production and distribution of INCAPARINA based on formula 9B, in Guatemala, El Salvador and Nicaragua. It is hoped that arrangements will subsequently be made for production in a number or the other countries in which protein malnutrition in children is an acute problem and which possess or can develop suitable local sources of cottonseed flour.

Discussion

The basic objective of INCAP's work with protein-rich vegetable mixtures for human consumption has been to develop a low-cost protein food suitable for feeding to young children which could be produced in technically underdeveloped areas at a price which families of limited economic resources could afford. There are many potential formulas, but for use in Central America, milk was both expensive and in short supply, soybeans were not grown in quantity and the prospect of producing a suitable quality of fish flour very doubtful. For a time sesame flour seemed promising but soon proved to be too expensive.

Cottonseed flour was employed cautiously at first in Vegetable Mixture 8 because of uncertainty about specifications for protein quality and possible adverse effects of its gossypol content. The favorable biological and clinical results with this formula encouraged INCAP to replace sesame flour entirely with the less expensive cottonseed flour. Sorghum was also substituted for part of the corn to further reduce the price and increase commercial flexibility.

The resulting formula for Mixture 9 represents a product in which the three principal factors of cost, nutritive value and acceptability were watched equally closely. The high nutritive value has been confirmed by very

extensive animal and clinical trials. Because it is also highly acceptable and cheap, INCAP Vegetable Mixture 9, depending on cottonseed flour for the quantity and quality of its protein, seems likely to have a considerable competitive advantage in comparison with comparable mixtures based on more costly protein concentrates.

The most serious limiting factor, the present lack of mills producing a sufficiently good grade of cottonseed flour, can be overcome through the application of existing technology. The INCAP results to date indicate that even when fed as 38% of a mixture furnishing the sole protein source of the diet, cottonseed flours containing levels of total and free gossypol in the range of 1.0, 0.04 and 0.06%, respectively, can have good nutritive values and produce no detectable adverse biological results, at least when the seed is processed in such a way that the quality of the protein is not damaged by heat.

While it is evident from the discussions of this meeting that more data are needed on the nature of the so-called "gossypol toxicity" in sensitive animals fed flours of poor quality, the results help to confirm the safety and effectiveness of cottonseed flours of high protein quality in which the initial seed does not contain excessive gossypol and is processed so that most of the pigment present is separated with the oil. Since cotton grows well in the tropical and subtropical regions where protein malnutrition is most common, the initial success of a vegetable mixture for human consumption containing 38% cottonseed flour, points to an important role for cottonseed protein in helping to meet the critical protein shortages of such areas.

Summary

INCAP Vegetable Mixture 8 containing 9% cottonseed flour and Mixture 9B containing 38% cottonseed flour, 58% whole ground corn and sorghum, 3% Torula yeast, 1% $CaCO_3$, and added vitamin A. have been found highly acceptable when prepared in the form of flavored and sweetened cereal gruels. Mixture 8 proved highly satisfactory in 15 children recovering from kwashiorkor and 5 with acute kwashiorkor, even as the sole source of protein. In 8 children fed from 2.3 to 3.5 gm. of protein per k. body weight nitrogen retention with milk averaged 19.1% of intake compared with 20.5% for Mixture 8 fed isoproteically.

Initial tolerance to Mixture 9B was established in children partially recovered from kwashiorkor; it was then used successfully in the treatment of 5 children with acute kwashiorkor. Plasma amino acids changes following a protein test-meal were studied in 6 children given either milk, Mixture 9B or a corn-bean diet for several weeks. The increase in plasma amino acid levels was essentially the same when milk or Mixture 9B constituted the protein source of the previous diet and much higher than that following the corn-bean feeding. Since this response bears a direct relationship to the protein value of the previous diet, the results are further confirmation of the similarity of Mixture 9B and milk in this respect.

In 9 children, 48 balance periods each on milk and Mixture 9B at a protein intake between 2.0 and 3.0 gm./k./day gave an average retention of 16.3% for milk and 17.8% for Mixture 9B. In 13 balance periods each at higher levels of protein intake, nitrogen retentions were still essentially the same for milk and Mixture 9B, although in 16 periods each at levels of 1.0 gm./k./day and below, retentions with milk were somewhat higher. No effect of fat on nitrogen retention was detected when it was added to either of the mixtures.

In field trials with Mixture 9B involving 115 children in Guatemala for 15-19 weeks and 53 in El Salvador for 4 weeks, approximately 95% liked the mixture and consumed it regularly. Preliminary results of acceptability trials in Honduras and Nicaragua are similar. In a marketing trial in Guatemala from March 22 to September 30, 1960, over 840,000 packages containing 75 gm. each of Mixture 9B at 3 cents each or their bulk equivalent were consumed with very favorable acceptability and no reports of intolerance.

The cottonseed flours employed were specially prepared for human consumption and contained over 50% protein of a relatively high biological value. They had approximately 1% total and 0.04 to 0.06% free gossypol. At no time was an intolerance or other adverse effect due to the cottonseed flour encountered even though it furnished nearly 80% of the dietary protein for periods ranging from a few weeks to 3 months.

Mixtures 8 and 9, and their several variations, have been given the generic name "INCAPARINA." On the basis of the extensive and favorable biological and clinical findings, they are recommended for the supplementary and mixed feeding of young children, and as low-cost protein-rich foods of good quality for persons of all ages in any area where the basic ingredients are available and where protein of animal origin is expensive or in short supply.

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DISCUSSION

Question: What is the selling price of the Vegetable Protein Mixture?

Scrimshaw: The selling price in Guatemala at the present time to non-profit instituitons is $15.00 a 100 lbs.—15 cents a lb.

Lyman: Have you used soybean products in your mixtures?

Scrimshaw: For the human work, we have not used soya in any of these tests because soya at the present time is not available in Central America; however, Dr. Bressani has used soy products in some of his animal studies—he may wish to comment.

Bressani: In studying supplementation of vegetable proteins, we find that at low protein levels of intake in the rat and in the chick, lysine is the most limiting amino acid. In order to improve the nutritive value of the mixture we can, of course, use lysine, but it would be better to use something cheaper than lysine. We have considered using 50% protein soybean meal in the mixture, but we haven't yet tested it for this purpose. We have added 3% soybean meal to the vegetable mixture to replace 3% of kikuyu leaf meal. The results showed that both growth and protein efficiencies were slightly improved. The addition of soybean meal provided more protein than the leaf meal and the improvement ob-

served was attributed to the higher amounts of lysine in this product.

Bradfield: Do you intend this mixture to be the only source of protein in the diet?

Scrimshaw: No. The whole purpose is to introduce a food that will become an important source of protein in the diet. I don't even want to call it a supplement. We hope that it will furnish a significant amount of good protein in the same sense that in the United States meat, milk and eggs supply protein. For experimental purposes we have used the mixture as the only source of protein or as the only source of all essential nutrients except ascorbic acid. A child or an adult does well on this mixture with nothing else but supplementary fruit juice and a calorie source, but this is not the purpose for which it is intended.

Bradfield: The reason I asked was I wanted to know why you adhere to a fixed 25% protein in the mixture if it is not the only source of protein in the diet. In cases where dietary surveys reveal low protein intakes, and the mixture is to be used as a supplement to the existing diet, it would appear reasonable to try and get an even higher precent protein say 30%, to combat the diluting effect of the existing diet.

Scrimshaw: It would get into too many considerations of pediatric practice and of existing food habits to answer in detail. Suffice it to say, however, the people in Central America are accustomed to consuming cereal gruels which they make from corn primarily, but also from other cereals. These form the bulk of many children's diets. They are very acceptable and the people don't realize that they are not of good nutritive value. The best way of improving existing diets seems to be to substitute for these local cereal gruels of poor quality, one of good quality. There are infinite varieties of ways of solving this problem. In some places, you may want a powder to add to soups, or powders to add to flours for enriching biscuits, etc. But this type of mixture fits into the cultural and economic situation found in Central America, in Mexico and in quite a few other parts of the world.

Lyman: How would this mixture apply to the United States? What would be the acceptability of this mixture in the United States?

Scrimshaw: Of course, it wasn't developed for the United States, but a group of physicians in Florida have become interested in the possibilities of using it for institutional feeding and also of making it available to elderly retired persons living on reduced incomes. They have carried out initial acceptability trials and have found it apparently quite acceptable in Florida for both purposes. On the basis of these findings, they have requested authorization to go ahead with a large-scale production and distribution in a number of states. Another group has found it highly acceptable among Mexicans and Americans of Mexican origin living in Texas. We also know that in Guatemala it has become quite popular among middle and upper income families. Furthermore, it has been used successfully by persons interested in losing weight. They can get all their nutrients from 4 glasses of INCAPARINA flavored with a teaspoonful of sugar per glass, and still consume only about 600 calories. We've actually had a young woman on our staff who weighed over 200 lbs.—lose 13 lbs. in 2 weeks. She continued to feel strong and comfortable, whereas on a variety of fadist diets she had always felt too badly and had given them up before losing this much weight.

Allison: Would you consider INCAPARINA a high protein cereal or consider it a protein food to be used as a regular dietary component particularly among low-income families?

Scrimshaw: It is, obviously, not an effort to pack all the protein possible into a mixture. This was the reason for my answer to Dr. Bradfield's question. We didn't want to put any more protein in than was needed because it costs money. We wanted something that would do a job economically and have practical value for mass use.

Question: What type of skim milk was used for comparison?

Scrimshaw: Most of the time it was a skim milk powder furnished us by the Nestle Company.

This introduces a point that should be discussed. When was the UNICEF reference milk established, Layton?

Allen: About 4 years ago.

Scrimshaw: The pertinence of this question is that there is not only binding of the lysine group with the heating of milk protein but also a slow deterioration in quality even in material in cold storage. In frozen storage, this is supposed to be negligible. Obviously, we have to question more than in the past—the quality of the protein in the milk used for comparison and even question how long a single batch of "standard reference skim milk" can continue to be used for this purpose.

Question: What are the specifications for the cottonseed flour?

Scrimshaw: The general specifications are those given in previous WHO protein advisory group documents with exception of the mesh. The grinding originally was done to pass 100 mesh but it was determined that 80 mesh gave a better consistency of product. Since we could get slightly more protein into the same volume of drink, the present specifications stand at 80 mesh for the corn, sorghum, and cottonseed.

Lyman: Is there any chance of this material being used for human consumption without cooking?

Scrimshaw: The answer is no. It's just not the kind of material that anybody would feed or would consume in quantity unless it were cooked. This raises a question as to the difference between specifications for the cottonseed flour as such and specifications for free gossypol in the final mixture. So far, we've stayed with specifications for the cottonseed flour, but it now looks more logical with the information which we have accumulated for INCAP to specify limitations on the amount of free gossypol and other substances in the total mixture.

Bradfield: What would happen if you put even more cottonseed flour in the mixture from the standpoint of cost and nutritive value?

Scrimshaw: We went from 9% of cottonseed flour in Mixture 8 to 38% in Mixture 9. The data already presented by Dr. Bressani show that not only would there have been no advantage in terms of protein quality in increasing further the amount of cottonseed flour, but that doing so might have resulted in some lowering of the protein quality.

THE DEVELOPMENT OF A LOW-COST HIGH NUTRITIVE VALUE FOOD SUPPLEMENT FOR PERUVIAN CHILDREN

by

Robert B. Bradfield

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U. S. Embassy, Lima, Peru

SUMMARY

The purpose of this project is to develop a practical low-cost high nutritive value food supplement for Peruvian children from foodstuffs available in this country. The Institute of Nutrition recently completed a 6-year study of Peruvian food composition (1)[1] and an 8-year program of dietary, biochemical and clinical surveys of nutritional status in representative urban and rural populations in the three geographical areas of the country (2). Nutritional studies have also been completed with pre-school children (3, 4, 5, 6, 7, 8). While some differences exist between the geographical zones, it may be said that the most widespread deficiencies found in these studies were quality and quantity of protein, vitamin A and riboflavin. Calcium intakes were low when compared to the National Research Council recommendations.

From basic crop production information

[1] Figures in parentheses refer to References at end of this article.

certain promising products were tested for their protein quality. Cotton is the largest cash crop. The Institute studied the quality of the press cake of several local producers as well as the high quality cottonseed flour sent by UNICEF. The practicability of producing a low fiber, high quality flour for human consumption was discussed with local producers. Cottonseed flour is particularly attractive not only as a rich source of high quality protein but also for the low cost. For these reasons, cottonseed flour is the principal ingredient of the mixture.

The protein quality of achita *(Amaranthus caudatus)* was also studied and found to be excellent (9). Achita is an inexpensive edible seed used widely in the sierra by the indigenous population. The high quality protein of quinua *(Chenopodium quinua)* has been previously reported by this laboratory (10). Habas *(Vicia faba)* are eaten in large quantities in Peru and have a higher lysine content than other beans.

Each of these foodstuffs was tested individually for general chemical composition and for the quality of its constituent proteins. This testing was accomplished by means of amino acid composition determined microbiologically and also by means of direct tissue repletion. A series of depletion-repletion tests with rats was carried out using casein as a control. From the results of these tests Supplement I was prepared and tested.

Alfalfa leaf meal was added for its high carotene (provitamin A) content. Torula yeast *(Candida utilis)* was added for its riboflavin contribution. The basic foodstuff of the area was added for palatability purposes. The ingredients were passed through a No. 40 screen to facilitate mixing. The supplement contains 26% protein and is low in fiber (2.5%) and fat (4.1%). Biological testing of the mixture with rats indicated growth qualities equal to or better than that of the casein control diet. Feed intakes and efficiencies were essentially the same as the casein controls. Possible toxicity is being studied by long term feeding over three generations of rats. The College of Veterinary Medicine is collaborating on the gross and histological examinations of specimens.

The taste is neutral and the supplement has little if any smell. The yellow color of the supplement, due to the cottonseed flour, is not a negative acceptability factor due to other yellow colors frequently found in Peruvian dishes from condiments. The cost of the supplement, on the basis of ingredients alone is less than $.05 U.S./lbs., or less than the cheapest staple, rice, the price of which is subsidized by the national government. This price does not include packaging and marketing costs however.

Supplement 2 represents a simplification of

Supplement I

A) **Ingredients**	
Cottonseed flour (UNICEF)	30%
Quinua	10%
Habas	10%
Achita	10%
Alfalfa Leaf Meal	2%
Torula Yeast	2%
Filler (wheat)	35%
	100%
B) **Composition (per 100 g)**	
Calories	352
Protein (Nx6.25)	25.7 g.
Fat (ether extract)	4.1 g.
CHO	55.3 g.
Fiber	2.5 g.
Ash	3.2 g.

Supplement II

Cottonseed flour (UNICEF)	35%
Quinua (or achita)	20%
Alfalfa Leaf Meal	3%
Torula Yeast	2%
Filler (wheat)	40%
	100%
Calories	352
Protein (Nx6.25)	26.3 g.
Fat (ether extract)	4.1 g.
CHO	54.0 g.
Fiber	2.5 g.
Ash	3.4 g.

Supplement 1. Habas was eliminated from the mixture due to the practical difficulty of removing the very hard outer skin prior to making the flour. Adequate quantities of lysine were supplied by adjusting the quantities of the remaining ingredients. To simplify the mixture further, 20% quinua was used in Formula 2-A and 20% achita was used in Formula 2-B instead of having 10% quinua and 10% achita as previously. The protein content and quality of achita and quinua are nearly identical so that the nutritional value remained the same. Cooking characteristics were altered considerably because quinua thickens on heating and achita does not. Human acceptability tests were carried out in a small jungle town with 15 families (100 persons) over a period of a year. The families consumed one to two kilos per family per week. During this time, the three different formulas were tested for acceptability and all were found to be acceptable on a long-term basis. By means of a mother's club in this town a number of different dietary preparations were made and tested in a cooperative fashion.

In preparing the mixture a pre-mix was made and then the staple of the area was added as a filler (coast-rice, sierra-wheat, jungle-yucca). The acceptability tests revealed, however, that the filler was not necessary from a taste standpoint. By eliminating the filler and adjusting the remaining proportions, the protein content of the mixture could be raised considerably. A final mixture of the same ingredients at a higher protein level is being calculated at the present time. This mixture will be tested in the school lunch programs, MCH centers, and on sick and well babies.

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Table I
Production Estimates of Leading Peruvian Crops [1]

Product	Area Cultivated		Production in Metric tons	Value in Soles [4]
	Hectars	% of total		
Cotton [2]	209,169	12.09	302,827	1'761,153,533
Rice	68,769	3.98	258,619	327,920,000
Quinua, Cañihua, Achita	34,360	1.99	37,768	58,540,000
Barley	194,185	11.22	225,802	164,835,000
Corn	231,561	13.39	303,836	297,600,000
Wheat	166,291	9.60	162,502	186,860,000
Bean	23,045	1.33	21,305	55,320,000
Chick pea and peas	21,000	1.21	18,900	47,200,000
Broad bean	22,000	1.27	21,890	19,700,000
Lentil	4,000	.23	4,000	11,600,000
Lima bean	1,467	.09	1,310	5,043,600
Sweet potato	10,169	.59	73,370	30,090,000
Oca, Olluco and Mashua (tubers)	26,021	1.50	93,270	60,625,000
Potato	245,602	14.20	1,453,118	1'364,000,000
Yuca	14,800	.86	201,441	143,388,000
Forage crops [3]	205,000	11.86	4,100,000	369,000,000

[1] Taken from Anuario Estadístico del Perú, Ministerio De Hacienda y Comercio, Lima, Peru, 1954.
[2] Of total cotton production in 1954, 60% was seed (174,290 metric tons).
[3] Ministry of Agriculture estimates over half above forage figure is alfalfa.
[4] 18 soles to U.S. dollar.

Table II
Nutrient Composition of Individual Components

Ingredient	Major Components (g/100 g)							Minerals (mg/100 g)			Vitamins (mg/100 g)			
	Calories	Moisture	Protein	Fat	Carbohydrate	Fiber	Ash	Ca	P	Fe	Vitamin A I. U.	Thiamin	Riboflavin	Niacin
Cottonseed flour [1]	355	11.27	52.00	5.50	24.47	0.30	6.08	228	1250	12.1	126	1.30	0.28	6.55
Broad bean flour	336	13.10	24.40	1.90	57.80	1.60	2.90	48	396	9.2	—	.33	.37	2.70
Achita	368	12.00	12.90	7.31	65.21	7.03	2.58	251	499	3.2	—	.13	.32	.96
Quinua	357	12.00	11.00	5.30	68.70	4.90	3.00	131	424	6.8	—	.52	.31	1.60
Torula yeast [2]	349	10.00	52.19	3.45	27.50	0.45	7.40	900	1950	17.5	—	120.00	40.00	100.00
Alfalfa leaf meal [3]	269	9.98	22.60	3.50	41.30	17.20	11.26	1600	300	21.5	31,996	.44	1.98	4.85
Wheat flour (Int. Ext.)	374	10.80	10.50	2.50	75.90	2.10	0.40	32	108	.3	16.7	.11	.06	.92

[1] UNICEF Code C-1
[2] Lake States Dried Torula Yeast (Type CF-4), Rhinelander, Wisconsin
[3] California Vegetable Concentrates Alfalfa Leaf Powder Huntingdon Park, California

Table III
Nutrient Composition of Supplement I

Ingredient	Major Components (g/100 g)							Minerals (mg/100 g)			Vitamins (mg/100 g)			
	Calories	Moisture	Protein	Fat	Carbohydrate	Fiber	Ash	Ca	P	Fe	Vitamin A I. U.	Thiamin	Riboflavin	Niacin
Cottonseed flour	106	—	15.60	1.65	7.34	0.09	1.82	68	375	3.6	38	.39	0.08	1.96
Broad bean flour	34	—	2.44	.19	5.78	.16	.29	5	40	.9	—	.03	.04	.27
Achita	37	—	1.29	.73	6.52	.70	.26	25	50	.3	—	.01	.03	.09
Quinua	36	—	1.10	.53	6.87	.49	.30	13	42	.7	—	.05	.03	.16
Alfalfa leaf meal	5	—	.45	.07	.83	.34	.22	32	6	.4	640	.01	.04	.10
Torula yeast	7	—	1.04	.07	.55	.01	.16	18	39	.3	—	2.40	.80	2.00
Wheat flour (Int. Ext.)	135	—	3.78	.90	27.32	.76	.14	11	39	.1	6	.04	.02	.05
Supplement I	360	9.06	25.70	4.14	55.21	2.55	3.19	172	591	6.3	684	2.93	1.04	4.63

Table IV
Nutrient Composition of Supplement II

Ingredient	Major Components (g/100 g)							Minerals (mg/100 g)			Vitamins (mg/100 g)			
	Calories	Moisture	Protein	Fat	Carbohydrate	Fiber	Ash	Ca	P	Fe	Vitamin A I.U.	Thiamin	Riboflavin	Niacin
Cottonseed flour	124	—	18.20	1.92	8.56	0.10	2.13	80	437	4.2	.44	0.45	.09	2.29
Quinua	58	—	2.20	1.06	13.74	.98	.60	26	85	1.4	—	.10	.06	.32
Alfalfa leaf meal	8	—	.68	.10	1.24	.52	.34	48	9	.6	960	.01	.06	.14
Torula yeast	7	—	1.04	.07	.55	.01	.16	18	39	.3	—	2.40	.80	2.00
Wheat Flour (Int. Ext)	150	—	4.20	1.00	30.36	.84	.16	13	43	.1	67	.04	.02	.04
Supplement II	347	8.44	26.32	4.15	54.45	2.45	3.39	185	613	6.6	1071	3.00	1.03	4.79

Table V
Amino Acid Content of Individual Components

Ingredient	Amino Acid Content (g/100 g)												
	Triptophan	Lysine	Methionine	Cystine	Isoleucine	Leucine	Valine	Arginine	Threonine	Phenylalamine	Histidine	Protein g	Nitrogen g
Cottonseed flour [1] (UNICEF Code C-1)	0.60	2.1	0.89	0.69	1.9	3.4	2.7	5.9	2.4	2.4	0.94	51.34	8.21
Broad bean flour	.19	2.24	.23	—	1.90	2.01	1.33	1.95	1.36	.99	.61	26.90	4.30
Quinua	.14	.80	.25	—	.82	.80	.48	.88	.56	.47	.36	11.81	1.89
Achita	.12	.92	.30	—	.77	.64	.59	1.10	.67	.49	.30	13.29	2.13
Alfalfa leaf meal	.47	1.13	.36	.59	1.08	2.31	1.38	1.20	1.47	1.22	.45	22.60	3.62
Torula yeast [2]	.72	4.33	1.02	.46	4.46	4.56	3.42	3.64	2.73	3.30	1.34	52.19	8.35
Wheat flour (Int. Ext.)	.13	.36	.20	.32	.62	.92	.58	.55	.39	.73	.29	12.00	2.11

[1] Food Research Laboratories, values supplied by UNICEF.
[2] Supplied by manufacturer.

Table VI
Amino Acid Content of Individual Components

Ingredient	Amino Acid Content (mg/gN)											
	Tryptohan	Lysine	Methionine	Cystine	Isoleucine	Leucine	Valine	Arginine	Threonine	Phenylalanine	Histidine	Protein Score
Cottonseed flour	73	256	108	84	231	414	329	719	292	292	114	75
Broad bean flour	44	521	53	—	442	467	309	453	316	230	142	36
Quinua	74	423	132	—	434	423	254	466	296	249	190	82
Achita	56	432	141	—	362	300	277	516	315	230	141	62
Alfalfa leaf meal	130	312	99	163	298	638	381	331	406	337	124	97
Torula yeast	86	519	122	55	534	546	410	436	327	395	160	66
Wheat flour	62	171	95	152	294	436	275	261	185	346	137	69
FAO Reference Protein	90	270	144	270	270	306	270	—	180	180	—	100

Table VII

Supplement I
Evaluation of Protein Quality

Ingredient	g.Nitrogen	Tryptophan	Lysine	Methionine	Cystine	Isoleucine	Leucine	Valine	Arginine	Threonine	Phenylalanine	Histidine
Cottonseed flour	2.46	180	630	267	207	570	1020	810	1770	720	720	282
Broad bean flour	.43	19	224	23		190	201	133	195	136	99	61
Quinua	.19	14	80	25		82	80	48	88	56	47	36
Achita	.21	12	92	30		77	64	59	110	67	49	30
Alfalfa leaf meal	.70	9	23	7	12	22	46	28	24	29	24	9
Torula yeast	.17	14	87	20	9	89	91	68	73	55	66	27
Wheat flour	.75	47	130	72	115	223	331	209	198	140	263	104
Total g.N	4.28											
mgAA/100 g		295	1266	444	—	1253	1833	1355	2458	1203	1268	549
mgAA/gN		69	296	104	—	293	428	317	574	281	296	128
Protein score		77	100	72	—	100	100	100	—	100	100	—

Table VIII

Supplement II
Evaluation of Protein Quality

Ingredient	g.Nitrogen	Tryptophan	Lysine	Methionine	Cystine	Isoleucine	Leucine	Valine	Arginine	Threonine	Phenylalanine	Histidine
Cottonseed flour	2.87	210	735	312	242	665	1190	945	2065	840	840	329
Quinua	.86	28	160	50	—	164	160	96	176	112	94	72
Alfalfa leaf meal	.11	12	31	9	16	29	61	37	32	39	32	12
Torula yeast	.17	14	87	20	9	89	91	68	73	55	66	27
Wheat flour	.84	52	144	80	128	248	368	232	220	156	292	116
Total g.N	4.85											
mgAA/100 g		316	1157	471	—	1195	1870	1378	2566	1202	1324	556
mgAA/gN		65	239	97	—	246	386	284	529	248	273	115
Protein score		72	89	67	—	91	100	100	—	100	100	—

Table VIIIa
Amino Acid Content of Premix I

Ingredient	(%)	g.Protein	g.Nitrogen	Tryptophan	Lysine	Methionine	Cystine	Isoleucine	Leucine	Valine	Arginine	Threonine	Phenylalamine	Histidine
Cottonseed flour	46.9	24.08	3.85	281	985	417	324	891	1594	1266	2767	1125	1125	441
Broad bean flour	15.6	4.20	0.67	30	349	36	—	296	313	207	304	212	154	95
Quinua	15.6	1.84	0.29	22	123	38	—	126	123	74	135	86	72	55
Achita	15.6	2.07	0.33	19	143	47	—	119	99	91	170	104	76	47
Alfalfa leaf meal	3.1	0.70	0.11	14	34	11	18	33	70	42	37	45	37	14
Torula yeast	3.1	1.62	0.26	24	145	34	15	149	153	115	122	92	111	45
	Total N		5.51											
	MgAA/100 g			389	1778	583	—	1614	2352	1795	3534	1663	1575	696
	MgAA/gN			71	323	106	—	293	427	326	641	302	286	126
	Protein Score			79	100	74	—	100	100	100	—	100	100	—

Table VIIIb
Amino Acid Content of Premix II

Ingredient	(%)	g.Protein	g.Nitrogen	Tryptophan	Lysine	Methionine	Cystine	Isoleucine	Leucine	Valine	Arginine	Threonine	Phenylalamine	Histidine
Cottonseed flour	58.3	29.94	4.79	350	1225	519	403	1109	1984	1575	3442	1400	1400	548
Quinua	33.3	3.93	.63	47	267	83	—	273	267	160	293	187	157	120
Alfalfa leaf meal	3.3	.75	.12	16	37	12	20	36	77	46	40	49	40	15
Torula yeast	5.0	2.61	.42	36	218	51	23	224	229	172	183	137	166	67
	Total N		5.96											
	MgAA/100 g			449	1747	666	—	1642	2556	1953	3958	1773	1763	751
	MgAA/gN			75	293	112	—	276	429	328	664	297	296	126
	Protein Score			84	100	78	—	100	100	100	—	100	100	—

DISCUSSION

Question: If you are stationed in Washington, how are you familiar with the situation in Peru?

Bradfield: I live in Peru and have been there the last 6 years. I have never been stationed in Washington. All testing referred to has been carried out at the National Institute of Nutrition of the Ministry of Public Health in Lima. Somebody asked me before about the figure for cottonseed cake production and I find that it was 10,000 tons in 1958.

Question: How much cottonseed flour is in the mixture?

Bradfield: 30% in Mix No. 1 and 35% in Mix No. 2.

Question: What is the quality of protein of Quinua?

Bradfield: The protein quality of quinua is excellent. Protein quality was measured by microbiological determination of amino acids, and by growth promotion and depletion-repletion tests with rats. The protein content of quinua runs 12-14% on an "as is" basis (about 12% moisture). The protein content of quinua is no higher than wheat or barley, but the protein quality is considerably better.

Question: Would the stability of products be a factor?

Bradfield: Quinua is sold in bulk in the market place; that is, one purchases by the handful from a sack. In preparing the mixture, we have purposely used quinua, achita, and habas from these markets. The products have been exposed to the ele-

ments for a considerable length of time.

Question: What is the composition of Mix 1?

Bradfield: No. 1, We have 30% cottonseed flour, 10% quinua, 10% Habas (Broadbean), 10% Achita, 2% alfalfa leaf meal, 2% Torula yeast, and a filler of 35% which in this case, was wheat. Supplement No. 2 is a simplification of the first mix on two counts: One, we temporarily eliminated the broadbean which gave us the difficulty of removing the hard skin, and secondly, we combined Quinua and Achita for the reason that the nutritive value is almost identical but they have very different cooking characteristics. In this mixture we have cottonseed flour, 35%; Quinua, or Achita, 20%; alfalfa leaf meal, 3%; Torula yeast, 2%; and wheat filler, 40%. We are reevaluating this filler possibility because we find that it is not necessary from an acceptability standpoint. We have tried feeding the premix and find that the fear we had that they would not accept it doesn't have much ground. We can change some of the ingredients if we desire from an acceptability standpoint.

Question: If the skin on the broadbean could be removed economically, would it be of value to the food supplement?

Bradfield: Yes. Habas is an inexpensive bean with a high lysine content, and good keeping qualities. As we saw in Table I, the production of habas is high and it is used as a human food in Peru, both toasted and in the form of a gruel. If we can find a simple way of getting rid of the skin without ruining the protein quality, it would be of value to the supplement.

SUPPLEMENTATION OF COTTONSEED PROTEIN WITH LYSINE

by

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Much of the poor growth of nonruminants fed rations containing cottonseed meal has been attributed to the gossypol content of the meals. Furthermore, gossypol has been held directly responsible for mortalities reported in some of the studies. Recent information lends further support to the belief that much of the poor growth is due to inadequate consumption and a deficiency of lysine rather than to any "toxic" manifestation of gossypol. The data have also indicated that L-lysine supplementation reduces the incidence of mortality attributed to gossypol. In the studies reported below, the term L-lysine is applied to both the crystalline, synthetic L-isomer of lysine and to fermentation products which supply L-lysine.

Effect of L-lysine Supplementation of Swine Rations on Growth

Dr. Lyman and Professor Hale (1, 3)[1] reported that L-lysine supplementation of cottonseed meal rations improved gains by as much as 500 percent when poor quality meals were fed. These and other results are shown in Table I. Pigs fed better quality meals supplemented with 0.3 percent L-lysine gained 60 percent more than unsupplemented groups. In another experiment by the same workers (1), a 33 percent improvement in gain was noted when 0.5 percent L-lysine was added to the cottonseed meal ration. A study reported by Dr. Hillier of Oklahoma State University (2) showed a 39 percent increase in average daily gains when 0.1 percent L-lysine was added to the cottonseed meal ration. In a trial conducted by the Ralston Purina Company (2), pigs supplemented with 0.25 percent L-lysine gained 30 percent more than the unsupplemented group. Work reported by Dow Chemical Company (2) revealed 40 to 65 percent increases in gains over unsupplemented cottonseed meal rations. In addition, Florida workers (1) reported improved gains of up to 113 percent when cottonseed meal rations were supplemented with L-lysine.

[1] Figures in parentheses refer to References at end of this article.

Table I
Results from Supplementing Rations With L-Lysine

	Ration	Feed Consumed lb.	ADG, lb.	% Increase Feed	% Increase ADG	Deaths	CM E-NH2-Lys g/16g.N
SWINE							
Hillier	CM113	2769	.75	—	—	0	1.6
	CM113+.1 Lys [1]	3123	1.04	13	39	0	
Lyman & Hale	L.Q.-CM Meal	200	.34	—	—	0	2.03
	L.Q. CM+.3 Lys	582	1.74	191	512	0	
	H.Q. CM	392	1.07	—	—	0	2.75
	H.Q. CM+.3 Lys	575	1.71	47	60	0	
Ralston Purina	CM112	556	1.06	—	—	0	3.7
	CM112+.25 Lys	655	1.38	18	30	0	
Dow	CM112	434	1.08	—	—	0	3.7
	CM112+.16 Lys	477	1.55	10	44	0	
	CM112+.37 Lys	527	1.77	21	64	0	
	CM112+.49 Lys	518	1.79	19	66	0	
Wallace & Aguirre	CM	196	.90	—	—	3	
	CM+.2 Lys	261	1.42	33	58	2	
	CM+.4 Lys	250	1.48	28	64	2	
	CM	35	.31	—	—	8 [2]	
	CM+.1 Lys	49	.46	40	48	8 [2 3]	
	CM+.2 Lys	61	.60	74	94	6 [2]	
	CM+.4 Lys	61	.66	74	113	4 [2]	

	Ration	g. Feed Consumed	g. Gain	% Increase Feed	% Increase Gain
CHICKS					
Deyoe et al.	CM	1600	605	—	—
	CM+.25 Lys	2500	1073	56	77
	CM	1197	347	—	—
	CM+.25 Lys	2074	768	73	121
	CM+.50 Lys	2101	1030	76	197
Scrimshaw & Bressani	CS flour	—	265	—	—
	CF+.2 Lys	—	427	—	61
	CF+.3 Lys	—	449	—	69
RATS					
Cabell & Earle	CM	—	98	—	—
	CM+.2 Lys	—	124	—	26
Scrimshaw & Bressani	CF	—	51	—	—
	CF+.1 Lys	—	60	—	18
	CF+.2 Lys	—	71	—	39
	CF+.3 Lys	—	62	—	22

[1] Lys refers to L-lysine.
[2] 31 of 32 pigs were alive at the end of the experiment but the deaths shown occurred in the two weeks following the experiment.
[3] Lived longer than unsupplemented group.

In all of the above experiments, lysine supplementation stimulated greater consumption of feed. But, more importantly, the lysine improved feed efficiency at an even greater rate. One example taken from the Florida experiments of Aguirre and Wallace (1) showed a 74 percent increase in feed intake when either 0.2 or 0.4 percent L-lysine was added to the rations. However, the 0.2 percent level of supplementation increased gains by 94 percent, whereas the 0.4 percent level increased gains by an additional 20 percent or 113 percent increase over the unsupplemented ration.

It is important to note that as the available lysine content of the cottonseed meal increases, as measured by the percent of lysine with free epsilon amino groups, the less is the response to supplemental lysine.

Effect of L-lysine Supplementation on Growth of Chicks

Deyoe *et al.* (3) reported recently that L-lysine supplementation of cottonseed meal rations increased gains by as much as 197 percent and feed consumption by as much as 76 percent. Thus, the same pattern of improved feed consumption and feed efficiency was noted with chicks as with swine.

Scrimshaw and Bressani (4) reported improved gains when INCAP Mixture 9′, containing 38 percent cottonseed flour, was supplemented with L-lysine. The 35-day gains were increased by 52 and 59 percent when 0.2 and 0.3 percent L-lysine, respectively, was added to the ration.

Effect of L-lysine Supplementation on Growth of Rats

Cabell and Earle have reported recently (1) a rat study in which 0.2 percent L-lysine supplementation of a corn-cottonseed meal ration resulted in a 26 percent increase in gains. When pure gossypol was added to the rations to a level of 0.04 percent, the L-lysine supplementation improved gains by more than 50 percent. However, the gossypol addition depressed gains in both the supplemented and unsupplemented groups by 28 and 14 percent, respectively.

Rosenberg and Culik (5) reported that the optimum performance of male rats fed a practical type, 14 percent protein, swine ration based on corn, cottonseed meal, and wheat flour middlings occurred when 0.6 to 0.7 percent lysine was added.

Scrimshaw and Bressani (4) reported improved gains when INCAP Mixture 9′, containing 38 percent cottonseed flour, was supplemented with L-lysine. The 14-day rat repletion gains for the 0.1, 0.2, and 0.3 percent levels of supplementation exceeded the gains of the unsupplemented group by 18, 39, and 22 percent, respectively. Addition of 0.1, 0.2, or 0.3 percent D,L-methionine did not improve the gains, but may have improved feed efficiency.

The Effect of L-lysine on Detoxification of Cottonseed Protein

Earle (6) reported that a 13-percent protein ration containing 0.011 percent pure gossypol was toxic to 2 of 7 pigs. However, there was no evidence of toxicity when L-lysine hydrochloride was supplemented at levels of either 0.1, 0.2, or 0.3 percent—even though the daily intake of free gossypol per kilogram of body weight was slightly greater than with animals not receiving supplementary lysine. These results were surprising to some investigators since lysine supplementation failed to prevent mortality in some North Carolina trials (1, 7). However, it is well to remember that the North Carolina trials involved the use of pigment glands as a source of gossypol, rather than pure gossypol. Furthermore, the level of gossypol supplied to the ration was three times that of the Beltsville work, but only 0.2 percent L-lysine was added. Unfortunately, the content of available lysine in the complete rations is unknown in both trials. It is important to note that no deaths occurred in the North Carolina experiment after feeding swine a ration containing auto-claved cottonseed meal and 0.026 percent gossypol supplemented with 0.2 percent L-lysine. However, one death occurred when no lysine was added.

Recently, Aguirre and Wallace (1) completed a study of L-lysine supplemented cottonseed meal rations. The data appear to confirm that L-lysine helps to protect against mortality. The pigs employed averaged only 10.6 pounds at the start of the experiment. It has been repeatedly shown that young, light pigs are very susceptible to toxic materials in cottonseed meal, but become less susceptible at older ages and heavier weights. In the

Florida study, all of the pigs died when fed the low lysine, unsupplemented ration or when only 0.1 percent L-lysine was added. However, when the L-lysine addition was increased to 0.2 percent, 2 of 8 pigs survived. When the amount was increased to 0.4 percent L-lysine, 4 out of 8 pigs survived. It is noteworthy that the feed consumption increased as the L-lysine supplementation increased up to 0.2 percent, but there was no further increase in feed consumption when this level of lysine supplementation was increased to 0.4 percent. However, the doubling of supplemental lysine to 0.4 percent caused a 20 percent additional increase in feed efficiency as well as a substantial reduction in mortalities.

In summary, L-lysine supplementation of swine, rat, or chick rations containing cottonseed meal or flour has resulted in greatly improved gains, feed consumption, and feed efficiency. Additional supplementation with D,L-methionine did not improve gains over lysine supplemented groups in many swine and chick trials, but may have improved feed efficiency. Furthermore, L-lysine supplementation appears to decrease toxic effects of cottonseed meal constituents under some conditions.

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DISCUSSION

Question: Would you comment on the work done in Arkansas and Mississippi in which quite high levels of lysine had been added and yet there was very little improvement or sometimes the added lysine was even detrimental. The cottonseed meal was of high quality.

Phelps: I cannot recall the Mississippi work, but in the Arkansas study I believe D,L-lysine was added. L-lysine supplementation has produced excellent growth increase, but the combination of D and L isomers of lysine has not been effective in several trials.

Lyman: We've seen quite an effect of lysine but I did want to point out that when we have a meal of really high protein quality, we can get gains, and we have obtained gains on several occasions equivalent to what you get with soybean meal. In other words, we obtain average gains of 1.7 to 1.8 lbs. per day with cottonseed meal as the sole supplement. I'm sure that if you supplement that kind of meal with lysine, the effect would be very, very little.

Question: What is the availability of high-quality cottonseed meals at the present time?

Aines: The high-quality cottonseed meal that was used in much of the amino acid work comparing soybean meals and cottonseed meals is not available at the present time. However, I feel sure that some of the other processing methods which are currently available or will be in the foreseeable future can produce high-quality meals. As far as current processes are concerned, I am talking about *some* prepressed solvent extraction meals and I certainly want to emphasize the *some* here because this is not a universal property of prepressed solvent extraction. On the horizon we see such things as the azeotropic extraction procedure that the Southern Laboratory is now developing. I think this method offers an excellent possibility for the production of exceptionally high-quality protein meals.

Scrimshaw: It should be pointed out in regard to these two figures that have been shown that a part of this effect of added lysine is not due to the need for more lysine in the cottonseed meal but to the fact that corn is also limiting in both tryptophan and lysine; so to some extent, the advantages of the higher quality of cottonseed flour used have been offset by combination with another material which is relatively low in lysine.

Frampton: Cottonseed meal 113 that is on this chart was the poorest meal available during that year. It is the one that we deliberately made poor by overheating. The other one, 112, was the best cottonseed meal that we could find throughout the nation. In 112 and 113, you have the extremes of commercial cottonseed meals.

Harper: You say that the 112 is the best that you could find—you *have* found what you considered better commercial meals in other years, is that right?

Frampton: Yes.

Phelps: Cottonseed meal 109 produced better gains and feed efficiency than cottonseed meal 112 in the North Carolina, Beltsville, and Oklahoma phases of the collaborative swine test. I would like to comment on Dr. Lyman's statement that we can produce as good gains with cottonseed meal as with soybean meal. In the swine trial at Dow Chemical Company, the gains with cottonseed meal fed pigs were superior to those receiving soybean meal.

Lyman: I was talking about cottonseed meal alone, without supplementation.

Phelps: The Dow trial employed cottonseed meal as the sole source of supplemented protein but with added amino acids.
In regard to best and worst cottonseed meal, how did we find the best and the worst? I didn't get them myself, but I know the worst was made that way by extreme heat treatment to reduce the available lysine content. That produced a very poor quality cottonseed meal.

Question: How did you know that this cottonseed meal was of poor quality?

Phelps: We determined this from the response of the animals. This was a large collaborative swine test involving 300 animals. We knew that the available lysine would be low because of the heat treatments. We expected a poor response from heat damaged meals.

Harper: Let's let Vernon talk for just about 2 minutes in regard to these meals. I think that it is important to know how these meals were selected.

Frampton: We received here in the Laboratory samples of meals from throughout the cotton-producing area. A quantity—I think it was 2 tons—of each meal was set aside and held until our analysis was completed. Then the cottonseed meals used in the swine experiment were selected on the basis of the epsilon amino lysine, on the basis of the total gossypol, and on the basis of the free gossypol. The combination of those three then served as an indication of what we consider to be the best and the worst of the cottonseed meals. Now, this 113 was from a meal that was very poor—we just made it a little worse. I won't make too much comment on this question of 109 and 112. This showed up at one of the tests where 109 was better than 112, but this wasn't consistent.

STATUS OF BREEDING FOR GLANDLESS (GOSSYPOL-FREE) COTTONSEED

by
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Cotton plants have glands containing darkly pigmented substances in all above ground parts. It is the gossypol in the glands within the seed that is of interest in this conference.

Hopi, an ancient cotton of the Hopi Indians of Arizona, was reported by Lewton in 1912 and by Fulton in 1938 to have variable numbers of pigment glands in the bolls. Hopi, however, is not a completely glandless cotton. McMichael at the U. S. Cotton Field Station, Shafter, California, crossed Hopi Moencopi with Acala. By selecting for glandless seeds among thousands of plants for several generations, he was able to develop a true breeding glandless seeded strain which had an extremely low, essentially zero, gossypol analysis.

As might be expected in a wide cross of this sort, the glandless strains developed during the early stages of this program were lacking in yield and fiber quality. But once the basic breeding stock was developed and once the inheritance of the character was on fairly sound grounds, the applied plant breeders were in a position to move ahead in combining glandless seed with the yield and quality required in a commercial variety. Seed stocks for breeding purposes have been released to many state, federal, and private breeders.

The most concentrated effort to develop glandless seed into a commercial variety has been at the U. S. Cotton Field Station, Shafter, California, where the glandless seeded line originated. Inheritance studies by McMichael showed that the glandless seeded condition was controlled primarily by two recessive genes. Purely genetic studies on the inheritance of glandless tissue in cotton is still underway, but enough is known for the applied breeder to proceed. With this information as a working hypothesis, Turner and Miravalle, the breeders at Shafter, began using the backcross method to transfer glandless seed into Acala 4-42 and a few of their most promising advanced strains. By using the cotton winter breeding garden at Iguala, Mexico, they were able to grow two backcross generations each calendar year. Thus, they proceeded quite rapidly, and their most advanced material is now in the 6th and 7th backcross stages. Six or seven backcrosses should be about enough unless the glandless genes are themselves deleterious or associated genetically with something harmful.

Whether the glandless seeded condition can be easily combined with the yield, quality, disease resistance, and other attributes of a successful commercial variety will not be known definitely until advanced glandless seeded strains can be thoroughly tested in field trials. Field testing necessarily must lag behind the most advanced backcross generation. Since glandless seeds depend upon recessive genes, backcross plants are glanded, but the ones which will produce a certain ratio of glandless plants following self-pollination can be recognized by reduced numbers of glands on the bolls. In order to test glandless seed lines for yield and quality, it is necessary to self-pollinate selected backcross plants, recognize pure glandless plants the following season, increase their seed supply to the amount needed for testing, and then conduct the test.

This year (1960) true breeding glandless strains extracted from the 2nd and 3rd backcross generations are being grown at Shafter in comparison with commercial checks. The selections look good, but they cannot be expected to measure up to Acala 4-42 in all features of yield, quality, and disease resistance. The 1960 tests will reveal how much improvement will be needed to produce a commercially acceptable glandless Acala. In 1962, sufficient seed should be available from strains extracted from the 6th and 7th backcross generations to make yield and spinning tests. This should determine whether the glandless genes are deleterious or associated with anything harmful. At present, there is no evidence to

suggest that this is likely.

In the early days of breeding for glandless cotton, stocks with a reduced gossypol analysis in the range of ¼ to ½ that of ordinary cotton were made available to breeders. Rhyne's glandless leaf stocks developed at Raleigh were among these, and the first stocks out of Shafter were not completely glandless. Materials derived from these early releases are still in many breeding programs around the country. At one time, some breeders believed that it would be easier to develop a variety with a reduced amount of gossypol than one with essentially zero gossypol. This may yet turn out to be the case, but experience seems to indicate that the completely glandless stocks now available are better basic breeding stocks than the early releases. Unless compelling evidence to the contrary develops, seeds with essentially zero gossypol should now be the breeding objective; and there is no reason to assume it would be a more difficult objective than a reduced gossypol content. Breeders who have stocks with reduced gossypol at various stages of development could start using glandless plants at this point in the breeding program. They may or may not feel that a fresh start would be to their advantage.

A majority of breeding programs, both institutional and private, across the country have glandless breeding programs underway to some extent. California is possibly further advanced, but the Delta Branch Station at Stoneville is about in the 3rd backcross stage in transferring glandless to a Deltapine and an Empire derivative. Recently the National Cottonseed Products Association, Inc., made grants-in-aid to both the Shafter and Stoneville stations to accelerate this work. C. A. Moosberg in Arkansas is in the 3rd backcross stage in the development of a glandless Rex and A. L. Smith in Alabama has initiated glandless breeding in the Plains variety. Several of the private seed companies have glandless breeding in various stages.

Interest and effort in glandless breeding is definitely increasing. If someone is able to breed and release a successful glandless variety, this undoubtedly will greatly stimulate others to follow suit. Although the price advantage of glandless seed has had no opportunity to be established in the trade, there undoubtedly would be a positive and direct benefit to the cotton farmer and hence to the entire industry. The value of the lint, however, so far overshadows the value of the seed that the breeders do not visualize that even a considerable increase in the value of the seed would offset an appreciable reduction in yield and quality of lint. Therefore, the task is to breed glandless cottons adapted to the different areas of production which are equal or superior to present varieties in other respects. This is a large order, but the importance of successfully accomplishing this goal justifies a vigorous effort.

REVIEW OF EPSILON-AMINO GROUPS AS A MEASURE OF AVAILABLE LYSINE

by
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SUMMARY

Rapid analytical methods to measure heat impairment of the nutritive value of proteins are needed. The first amino acid to be altered by heat processing of proteins is lysine. Accurate determination of lysine in a protein before and after processing should therefore allow to evaluate heat damage in proteins. In order to test this, a series of milk powders, having been submitted to different heat treatments, were analyzed for lysine according to 4 methods, namely:

(a) lysine content after acid hydrolysis according to Moore and Stein or Gale

(b) lysine liberated by digestive enzymes *in vitro* according to Mauron

(c) lysine with free ε-amino group according to Carpenter

(d) lysine available to the rat *in vivo* according to Harper

The results of this comparison show that (b) agrees perfectly with (d). The next best agreement is found between (c) and (d), whereas (a) correlates very poorly with (d). The following explanation may be given for this finding:

During heat treatment the free ε-amino groups in the protein react with carbohydrates, always present in unpurified proteins and foods. The so-formed sugar-amine linkage is resistant to the attack by the digestive enzymes, whereas acid hydrolysis is able to split it, thus regenerating the free ε-amino groups. Usual lysine determination after acid hydrolysis yields therefore too high results, which do not represent the true amount of lysine actually available in the heated protein. On the other hand, since the fluorodinitrobenzene (F-DNB) reagent, used to detect free amino groups in proteins is applied prior to acid hydrolysis, the ε-amino groups linked to sugars are not determined, so that the lysine value brought forth by the F-DNB-method represents available lysine in much the same way as the lysine value obtained by in vitro enzymic digestion does. The latter procedure presents, however, some difficulties with vegetable proteins, so that in this case the F-DNB-technique should be the method of choice.

Carpenter's modification of the F-DNB-method then was described and some results in the evaluation of the protein quality of peanut flour were presented. Sources of error, due to the reduction of aromatic nitro groups during acid hydrolysis of the dinitrophenylated protein in presence of reducing sugars were discussed.

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DISCUSSION

Question: How did you standardize the enzymes?

Mauron: We standardized the enzymes for the whole procedure by using a standard product for digestion which was lyophilized milk powder or a reference spray-dried milk powder of top quality giving the same performance on in vitro digestion. The reference milk powder was kept under refrigeration. It was always used as standard of reference for the testing of milk preserves. The enzymes were standardized commercial enzymes preparations, but even so differences were observed from batch to batch. Each batch was therefore standardized with the reference milk powder.

Question: What differences in available lysine do you have under different conditions of storage of milk powders and vegetable proteins?

Mauron: We have only some experience with milk powders in storage, because we did not do any systematic study of vegetable proteins in this respect. The stored vegetable proteins we analyzed were generally kept in sealed cans under refrigeration (—5 to —8° C). Under these conditions we could not find any changes for, let us say, 3 years in the peanut flours. When milk powders are stored at 37° C in open cans at a relative humidity of 90% you get a very rapid loss of available lysine which reaches about 70% in 2 months.

There are some classical investigations of Lea and Hannan, made at Cambridge about 10 years ago, on the storage of milk powder at 37° C and very high humidity. We also may get sometimes a reduction of lysine availability in sweetened condensed milk upon improper storage in tropical countries, although in fresh or properly stored sweetened condensed milk lysine availability is very high (95 to 100%), higher indeed than in evaporated milk (80%). It is easy to detect this damage on storage, because of the brown color of the milk. This is not a good measure, of course, but an indication that something has happened. I remember one case of a shipment of sweetened condensed milk to Malaya, where the milk was apparently stored in the ship near the engine. We do not know exactly what the temperature was—but on arrival some samples had turned brownish in color. Lysine deterioration in such caramelized milk may reach 50% or so.

Unfortunately, we have no experience on the loss upon storage of vegetable proteins, but the situation is probably about the same, with, perhaps, a quantitative difference. Certainly, storage under humid conditions has to be considered dangerous to lysine in any case.

Question: Is it possible to obtain details of your methodology for available lysine?

Mauron: It has been published in Archives of Biochemistry in 1955 (Vol. 59, 433-451).

Question: How was the milk dried?

Mauron: All milk powders showing a loss in lysine availability were drum-dried.

Altschul: I would expect that changes in vegetable protein flours would be similar to those in milk.

Mauron: Yes. In principle it is the same but quantitatively you can expect, because you have less reducing free sugars, that the damage would be less severe. But it is to be said that lactose is not the most active sugar in Maillard reaction by far. For instance, pentoses are much more active; that was a point found by Carpenter in fish flour. Because fish powder doesn't contain much sugar, people thought white fish flour would be free from damage. But there are small amounts of pentoses and they are very active in the Maillard reaction so that the low quantity of sugar is compensated by the more active sugar.

RELATIONSHIP BETWEEN COTTONSEED CONSTITUENTS AND PROTEIN VALUE-EPSILON-AMINO-FREE-LYSINE OF COTTONSEED PROTEINS

by

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We in the Southern Regional Research and Development Division are interested in forming a definition—a quantitative definition in terms of cottonseed constituents—of a cottonseed meal that has the highest possible nutritional quality. Some progress has been made in this direction, and we are now able to give a fairly precise definition of such an ideal meal; a cottonseed meal with 0.0% total gossypol and at least 4.2 grams of epsilon-free amino lysine per 16 grams of meal nitrogen will produce excellent growth when it is used as the only protein supplement in corn rations for broilers and swine.

This definition developed from a series of studies involving a large number of commercial and laboratory-prepared cottonseed meals. These meals were used as the protein supplement in corn rations and were fed in a series of tests to over 12,000 broilers and 300 pigs. Analyses obtained from these tests are the subject of this discussion.

In one series of experiments, the rations were mixed at a central location and were shipped to the several stations where they were fed to broilers. The weight of the chicks

and their feed consumption data were obtained at the end of 4 and 8 weeks. A ration containing soybean meal served as the reference ration in these tests.

The agreement of the results among the four stations selected for the present analysis may be seen from the data in Table I. Here the ratio of the weights of the broilers on a cottonseed meal to that on the soybean ration served as an index of the nutritive quality of the cottonseed meal for use in the subsequent computations. The correlation coefficients of these indices between the stations were calculated, and the coefficients are recorded in Table I. It will be noted that the correlation coefficients are large, and therefore, the agreement between the stations is excellent.

Table I
Correlations of Nutritive Indices Between Stations

WEIGHT AT 4 WEEKS

Stations Compared	Meals in Common	Correlation Coefficient
0-3	5	0.994
0-5	5	.971
0-9	5	.988
3-5	6	.981
3-9	6	.992
5-9	7	.997

Extensive chemical data were obtained on each meal, and multiple linear regression analyses of growth on these chemical constituents of the meals were carried out. The total correlations calculated between growth and individual chemical constituents of the meals led to the suggestion that, of the many constituents determined, the only factors of interest were the epsilon-free amino lysine, the total lysine, arginine, the total gossypol, the crude fiber and the energy contents of the rations (Table II).

Table II
Correlations Between Chemical Constituents and Average Weight of Broilers

4 WEEKS—STA. O

Constituent studied	Concn. range	Correlation with Wt.
Lysine	0.468-600	0.997
Arginine	1.82-2.07	.561
Total gossy.	.222-407	—.678
Free	.006-.097	—.106
Crude fiber	4.31-6.36	.250
Calories added	1008-775	—.426

While it is recognized that total correlations may be completely misleading, attention is limited in this discussion to an assessment of the chemical constituents listed above. Moreover, the attention is limited to one station, namely station 0. The limitation of the computations to one station is justified on the basis of the correlations found in Table I.

The meals used in this test were all commercial meals, and the variation in the epsilon-free amino lysine from meal to meal was not great enough to enable us to assess its influence with sufficient precision. Therefore, the analyses involving the epsilon-free amino lysine are not included in these computations for this set of experiments.

The analysis of variance of the regression of growth on lysine at 4 weeks is reported in Table III. The F value of 61 with 4 and 1 degrees of freedom indicates that the regression is very highly significant.

Table III
Analyses of variance of multiple regressions of average weight of broilers on lysine, on lysine plus total gossypol, on lysine plus total gossypol plus crude fiber.

Variance	SS	df	ms	F
Lysine alone				
Due to reg.	14,931	1	14,931	61
residual	983	4	245	
Lysine plus gossypol				
Due to reg.	15,209	2	7,605	32
residual	706	3	235	
Lysine plus gossypol plus crude fiber				
Due to reg.	15,225	3	5,075	15
residual	690	2	345	

We found no increase in the F ratio when the multiple regression of growth on lysine and total gossypol was carried out. The F value calculated was only .32 with 3 and 2 degrees of freedom. Apparently the test is not precise enough to measure any influence

of total gossypol on the growth of the broilers.

We also found no increase in the F ratio when the multiple regression of growth on lysine plus total gossypol plus crude fiber was calculated, as may be noted in Table III. The F value is only 15 with 2 and 3 degrees of freedom. Apparently the test is not precise enough to measure any influence of crude fiber plus gossypol on the growth rate of the broilers.

It is not possible with the data available to carry out the analysis of variance of the regressions where more variables are considered. This type of analysis is limited because of the number of cottonseed meals used in the study being not great enough for a comprehensive study. However, an assessment of the confidence one may have in the partial regression coefficients in the regression equation may be made. The regression equation for the regression of weight gain in 4 weeks (W) on lysine contributed by the cottonseed meal (L), the total gossypol content of the ration (g), the crude fiber contributed by the cottonseed meal (z), and the arginine also contributed by the cottonseed meal (A), is given:

$$W = K + (1142 \pm 377)L - (126 \pm 166)g + (1.0 \pm 43)z + (1 \pm 137)A$$

In the above equation, the confidence limits, for odds of 20:1, are ± 377 for the coefficient for lysine, ± 166 for the coefficient for gossypol, ± 43 for the coefficient for crude fiber, and ± 137 for the coefficient for arginine. It is evident from this equation that the contribution of gossypol, crude fiber, and arginine are too small to be measured, and there is no reason for assuming that the coefficients for gossypol, crude fiber, and arginine are different from zero.

The computations for the regression with five independent variables (where the energy content of the rations is also included) have not been completed. Of all of the chemical constituents studied, only the variation in the lysine (contributed by the cottonseed meals) among the rations contributed to the variations among the average weights of the broilers on those meals. In other words, the growth response was directly proportional only to the lysine of the cottonseed meals.

The response of swine is comparable to that of broilers, in that the effect of total gossypol (when the growth of the experimental animal is used as the sole indication of the nutritive quality of the meal) is also too small to be measured. A series of 8 meals were used as the source of the protein supplement in feeding tests with 8 litters of 8 weanling pigs each, and the weights were recorded when the pigs had been on the rations for 270 days. Analyses of variance of the regressions of weight of pigs on the epsilon-free amino lysine in the meal proteins and of the weight of the pigs on the epsilon-free amino lysine and total gossypol are reported in Table IV. Note that the F ratio for lysine alone is 13—this for 6 and 1 degrees of freedom is of a high order of significance. Note also that the F ratio was only 6.5 for 5 and 2 degrees of freedom when gossypol was included in the analysis.

Table IV

Analyses of variance of multiple regressions of average weight of swine on epsilon-free amino lysine and on epsilon-free amino lysine and total gossypol

SWINE TEST

Variance	SS	df	ms	F
E-Lysine				
Due to reg.	7761	1	7761	13
about reg.	3077	6	512	
E-Lysine & total gossypol				
Due to reg.	8112	2	4028	6.5
about reg.	3094	5	619	

It was established by Conkerton and Frampton that gossypol adds to the epsilon amino groups of proteins. The evidence they presented is that the number of such groups in each of a series of proteins that will react with a 2,4-dinitro-fluoro-benzene is reduced if the protein is first subjected to reaction with gossypol. It was also established (Martinez and Frampton) that lysine in cottonseed meal proteins is destroyed when the meal is heated.

Use was made of these two observations to demonstrate that the protein depleted rat is unable to utilize cottonseed meal lysine that is bound with gossypol (Martinez & Frampton). Glandless cottonseed served as a source of cottonseed protein that had never been exposed to reaction with gossypol. The seed was hulled, rolled into flakes and defatted with hexane. A portion of the meal was then extracted with 80% ethanol for the purpose of

removing the sugars present. The fat-free meal and the de-sugared meal then served as stock sources of meal for other treatments. Gossypol (1% the weight of the meal) was added to one portion of the de-sugared meal, while raffinose (10% the weight of the meal) was added to a second portion. Both gossypol and raffinose were added to a third portion. One portion of each of the meals listed above was heated in a steam-jacketed autoclave at 121° C for 20 minutes, and a second portion was heated in the same autoclave at 121° C for 60 minutes. Every meal was then exhaustively extracted with diethyl ether and used as the source protein in a rat depletion-repletion test. In addition, the total lysine (ion exchange) and the epsilon-free amino lysine (lysine that reacts with 2,4-dinitro-F-benzene) were determined.

The coefficient of correlation between the total lysine and weight gain was found to be 0.49, with 16 degrees of freedom, while that between the epsilon-free amino lysine and weight gain was 0.89 also with 16 degrees of freedom.

Analyses of variance of the regression of weight gain on total lysine destroyed by the treatment and of the regression of weight gain on the amino lysine found are shown in Table V.

Table V

Analyses of variance of weight gain of protein depleted rats on destroyed lysine and on bound lysine.

Variance	SS	df	ms	F
GLANDLESS COTTONSEED MEAL				
Lysine Bound				
Due to reg.	1563	1	1563	26
about reg.	931	14	66	
Lysine Destroyed				
Due to reg.	388	1	388	2.6
about reg.	2106	14	150	

Note that the F ratio for the regression of weight gain on lysine destroyed is only 2.4, for 14 degrees of freedom. This regression is not significant, even for odds of only 10:1. On the other hand, the F ratio for the regression of weight gain on the bound lysine is 26, which is very highly significant. Evidently the protein depleted rat cannot use lysine in cottonseed meal proteins if the epsilon amino groups are bound by gossypol. Thus, when weight gain is used as the only criterion of nutritive quality, the epsilon-free amino lysine of the cottonseed protein constitutes an excellent means of chemically assessing the meal quality.

Although there is a high negative total correlation between the total gossypol and the growth response, this correlation is misleading, for when the epsilon-free amino lysine and total gossypol are both used in the regression analysis, it is found that the partial regression coefficient for total gossypol is probably zero. Also, in analyses of variance of these regressions, it is evident that the total gossypol does not contribute to the total variance, the same answer is obtained irrespective of whether the total gossypol is included or excluded from the computations.

None of the data obtained in the studies reported here supports the view that the rate of growth of an animal is a function of the free gossypol content of the cottonseed meals fed. Some may wish to assume, from the data reported, that gossypol in cottonseed meals has only the effect of reducing the amount of lysine that is available to test animals. It must be stressed, however, that THE ONLY CRITERION USED IN THIS STUDY WAS THE GROWTH RESPONSE OF THE TEST ANIMALS. It would be reckless for any one to promote cottonseed meal as a source of human food on the assumption that the only effect of gossypol is that of binding lysine. We know gossypol to be toxic, and we know that there are gossypol derivatives in cottonseed meal that are physiologically active. Evidence that this is so is found in the discoloration of the yolks of stored shell eggs produced by hens fed cottonseed meal. Evidence is also found in the mortalities of embryos in eggs produced by hens on cottonseed meal-containing rations. Evidence is also found in the observations that gossypol has been isolated from livers of swine receiving cottonseed meals. Also, there is damage, due to gossypol, of kidneys of swine and rabbits receiving cottonseed meals. It is the feeling of the author of this report that cottonseed meals containing high levels of total gossypol may not be fed with impunity in the long time use of cottonseed meals as a source of protein for human beings.

An added reason for suggesting caution in

the promotion of cottonseed meals (as they are produced commercially at this time) for human food is found in the unexplained mortalities that occur among swine receiving certain cottonseed meals. While we do not know the reasons for the deaths of the pigs, the evidence available very strongly supports the suggestion that precautions be taken with meals that are to be fed to human beings to reduce the crude oil to a low level, and to restrict the selection of meals to be used to those with low total gossypol contents.

DISCUSSION

Question: Are there other methods for determining epsilon amino lysine that are promising?

Frampton: There is a possibility that we have not explored of going back to the old Van Slyke as the method for measuring lysine. I don't know if it would be any good; I don't know if it would correlate any better. The method that we have been using for epsilon amino lysine is somewhat cumbersome. There is a lot of questions about the precision of the method, although the workers in this laboratory, Miss Carter for example, can reproduce their results excellently. Others seem to have trouble with it. I think that it's possible to improve upon this method and this we are undertaking in the immediate future.

Mauron: We did some experiments with the Van Slyke method after enzymic hydrolysis; there is a correlation between the decrease of amino nitrogen and that of available lysine during in vitro digestion of milk, but it is somewhat erratic. Enzymic "available lysine" values correlate much better with the chemical "available lysine" values according to Carpenter. I do not think that the determination of available lysine using Carpenter's method is really so complicated. We use it in a routine manner; a good technician, once she is used to the method, can run several samples a day. I cannot see how you could develop an easier method, for the time being, giving the same excellent correlation and being theoretically sound.

Frampton: I don't know; I would be rather skeptical about them because you are not measuring lysine in this case but you are measuring other products, and the reaction probably is not stoichiometric.

Mauron: In the investigations on peanut flour we also studied the availability of methionine and its possible correlation with lysine availability. Obviously, methionine availability could only be determined by in vitro digestion, since there is no chemical method for "available methionine." We could not find any significant differences between the peanut flours studied. In an overheated peanut flour, where lysine availability was reduced, methionine availability was also diminished, but it was within the error margin of the enzymic method for methionine which is rather high.

Question: Is there a correlation between the lysine availability and the protein solubility?

Frampton: The answer is yes, there is a correlation. However, the correlation between the growth response of animals and lysine is much better than between protein solubility and growth.

Lyman: Solubility, of course, is highly empirical. When we take meals prepared by greatly different methods, particularly chemical procedures, we're likely to get quite erroneous values with nitrogen solubility. If we take prepress solvent meals or screwpress meals, we can pick out those outstanding meals without question with nitrogen solubility. For a specific type of processing and for the purpose of picking out the outstanding meals, I would put just as much reliance on solubility as on total lysine or lysine epsilon amino groups. Where we get any problems is in those intermediary ranges where nitrogen solubility may not always be as accurate a method of measuring the nutritive value of the protein.

Altschul: I don't want to ask a question, I want to comment on Carl's statement—I must say that I disagree with him com-

pletely. I think that there is a basic issue here involved and I would like just to discuss that point. I think that it is difficult to try to do something *in vitro,* by chemical methods, that will in any way correspond to the very complex digestive processes that take place in the gut. At best, you're in a tough spot to try by one single measurement to come to some sort of a relationship with what is happening in real life. Therefore, the best you can hope is to pick a measurement that makes some sense. There is nothing nonsensical about nitrogen solubility—don't get me wrong, but I think that you have to try to get some analogy that would somehow make some sense to what's happening in the gut. As Dr. Mauron pointed out on several of his slides, you can see a connection between the binding of lysine to render the epsilon group unavailable chemically to what might happen to blocking a group so that it is not digested by trypsin or any of the other enzymes. That makes sense. You can suspect that there would be a relationship between what you get by this kind of a chemical measurement and what you get by real life behavior; but when you are talking about solubility, there really is no relationship. You can cook meat, you can take proteins and make them entirely insoluble and still they are just as nutritious because the factors which have to do with protein solubility are not necessarily factors that relate to digestibility. I quite agree with Carl that if you take a specific set of circumstances, you are going to get a correlation between nitrogen solubility and performance. But it is a correlation based on accident and not based on any real relationship between that subject and digestion.

Lyman: In answering that, I'm going to tell you about some other work on the relationship of lysine epsilon amino groups. This we have done in the laboratory; we have combined the epsilon amino groups with glucose with extensive reduction in the lysine epsilon amino values without any reduction in nutritive value. This depends on *how* your carbohydrate is combined. There is a difference, and so this too has a limitation. I don't think it's a limitation which we need to be concerned about in picking out the superior meal. I have a great deal of confidence in the determination of lysine epsilon amino groups, but this relationship is not perfect. It's quite easy, if we control the moisture content, to get the carbohydrate combined with this lysine epsilon amino group and have a very digestible product and yet very low lysine epsilon amino groups. I do not think this happens in commercial processing.

REVIEW OF THE RELATIONSHIP OF GOSSYPOL TO PROTEIN QUALITY

by
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The recognition that the chemical combination of gossypol with protein molecules during processing is an important factor in determining the nutritional value of the protein for non-ruminant animals was a major advance in cottonseed meal research. Much of the evidence for this relationship is to be found in the collaborative studies which this group has conducted during a number of years.

In an early collaborative study, which included both experimental and commercial meals, it became apparent that the best meals never had high levels of bound gossypol and the poorest meals, as a group, did have high levels of bound gossypol. Some of these data, which are recorded in the literature, are shown in Table I. In addition to the chick growth rate data, Table I also shows a marked reduc-

tion in lysine availability with increased bound gossypol content. This finding definitely pointed to protein quality.

Table I
Total Gossypol Content and Nutritive Value of Cottonseed Meals [1]

Meal No.	Total Gossypol	Chick Growth Rate Index [2]	Lysine Availability
	%		
10	0.15	100.0	80.7
16	.62	102.4	
15	.63	99.1	82.6
13	.76	97.1	76.9
14	.66	93.8	
9	.79	79.0	
19	.84	67.0	
11	1.02	66.7	
12	1.11	61.4	
29	1.15	58.3	
20	.95	56.0	
18	.87	54.9	
5	1.18	52.2	
24	1.17	50.0	68.1
22	.94	44.6	55.3
25	1.00	43.2	
3	1.30	43.0	
4	1.28	39.7	
1	1.34	39.3	
2	1.32	38.4	
23	1.11	30.8	53.2
21	1.11	22.7	50.7

[1] From the J. Nutrition, 49, 687 (1952).
[2] Average values for two assays.

Table II shows the results of a later collaborative test with prepress solvent meals. The same relationships are apparent here.

Table II
Protein Quality and Chemical Characteristics of Prepress-Solvent Cottonseed Meals from Different Mills

Mill designation	No. of samples	Chick growth rate index	Total Gossypol	Nitrogen Solubility in 0.02 N NaOH	Gossypol Content of cottonseed kernels [1]
			%	%	%
D	2	75.2	0.72	81.6	0.65
H	3	74.6	.84	77.6	.74
I	3	74.6	.73	77.0	.67
E	3	69.5	.85	74.1	.67
J	3	71.8	.81	70.0	.69
A	1	52.1	.86	68.5	.69
F	3	64.3	1.05	69.2	.81
B	3	59.9	1.06	67.9	.91
C	2	54.6	1.02	65.8	.76
G	3	51.8	1.23	68.8	1.01

[1] Rolled cottonseed meats used in the preparation of the meals.
J. Amer. Oil Chem. Soc., XXXII, 103 (1955).

Correlation coefficients between total gossypol and protein quality as determined by various types of feeding trials conducted by investigators in industrial laboratories, U.S.D.A. research laboratories, and state agricultural experiment stations are given in Table III.

Table III
Correlation Coefficients Between Total Gossypol and Nutritional Value as Determined by Various Types of Feeding Trials

Meal Series	Type of Test	Group Conducting Investigation	Correlation Coefficients
Prepress solvent cottonseed meals used in collaborative test. [3]	Chick Growth Rate	Texas	—0.899 [2]
		California	— .791 [2]
	Protein Efficiency Index (Chick Test)	Louisiana	— .40 [1]
		California	— .805 [2]
	Rat Protein Repletion	U.S.D.A.	— .682 [2]
Meal used in collaborative broiler experiment Series 1	Chick Growth Rate	Arkansas	—0.678
		L.S.U.	— .663
		Buckeye	— .767
		Beltsville	— .698

[1] Significant at the 5% level.
[2] Significant at the 1% level.
[3] J. Amer. Oil. Chem. Soc. XXXII, 103 (1955).

The next step was to show that when gossypol combines with protein, even without heat, protein quality and more specifically lysine availability are markedly reduced. This is shown in Table IV.

Table IV
Change in the Nutritional Value of Cottonseed Protein on Reaction with Gossypol

Sample description	Bound gossypol	Nitrogen Solubility in 0.02N NaOH	Lysine Availability [2]	Rat protein repletion value [1] Av. Gain in wt. in 10 days
	%	%	%	g.
Original protein	0	89.0	82.9	51.0
Methanol-treated protein	0	85.5	86.0	49.4
Gossypol-protein complex	3.25	48.3	48.7	16.6

[1] Five rats in each group.
[2] Three rats in each group.
Reprinted from The Journal of the American Oil Chemists' Society, January 1957 Issue, Vol. XXXIV, 21.

It became necessary to show that when bound gossypol is removed from cottonseed meal, protein quality and lysine availability goes up. The experimental evidence is given in Tables V and VI. In later studies, it was shown that as gossypol combines with the protein, the content of lysine with free epsilon amino groups progressively decreases.

Table V
The Effect of Removing Bound Gossypol on Protein Quality as Determined by Rat Protein-Repletion Test [1]

Description of meal	Av. initial wt.	Av. final wt.	Av. gain in wt. in 10 days
	g.	g.	g.
Original cottonseed meal	113.8	139.6	25.8
Treated to remove free gossypol	114.4	143.6	29.2
Treated to remove bound gossypol	118.0	163.8	45.8
Standard meal (butanone-extracted)	116.6	171.6	55.0

[1] Five rats in each group.
Reprinted from The Journal of the American Oil Chemists' Society, Vol. XXXIV, 21. January 1957 Issue.

Finally, evidence concerning the mechanism by which bound gossypol reduces protein quality was obtained. Table VII shows that when gossypol combines with protein, the action of the proteolytic enzyme pepsin is partially blocked. Table VIII shows that the hydrolytic action of trypsin is similarly affected. This interference with the action of the proteolytic enzymes is to be expected since the binding of gossypol to a protein reduces free epsilon amino lysine.

Table VII
Peptic Digestion of Cottonseed Protein After Various Treatments

	Digestion time in hours 0	2	4	8	24	% reduction in digestion
	mg. amino nitrogen/100 ml. digest [1]					
Original Cottonseed Protein no treatment	3	22	37	48	62	
Autoclaved 1 hour	3	23	39	50	61	1.6
Autoclaved with glucose 1 hour	5	24	41	53	62	0
Autoclaved with gossypol 1 hour [2]	3	20	24	38	41	34
Treated with gossypol in methanol at room temperature [3]	2	16	18	23	39	37

Sample weights were adjusted to give 0.9 g. of nitrogen in each test.
[1] Each value represents an average of three enzymatic digestion trials.
[2] Bound gossypol content, 1.30%.
[3] Bound gossypol content, 1.38%.

Table VIII
Tryptic Digestion of Cottonseed Protein After Various Treatments

	Digestion time in hours 0	2	4	8	24	% reduction in digestion
	mg. amino nitrogen/100 ml. digest [1]					
Original Cottonseed Protein No treatment	5	24	30	39	40	
Autoclaved 1 hour	4	24	36	39	40	0
Autoclaved with glucose 1 hour	5	16	21	24	30	25
Autoclaved with gossypol 1 hour [2]	2	12	13	20	28	33
Treated with gossypol in methanol at room temperature [3]	2	8	9	12	17	58

Sample weights were adjusted to give 0.9 g. of nitrogen in each test.
[1] Each value represents an average of three enzymatic digestion trials.
[2] Bound gossypol content, 1.30%.
[3] Bound gossypol content, 1.38%.

The success which Dr. N. S. Scrimshaw has reported on the use of cottonseed flour in human nutrition calls attention to the need for additional information concerning the effect of boiling cottonseed flour with water on nutritional value and chemical caracteristics.

Table IX shows the results of tests designed to obtain some of this information. The interpretation of these data is as follows:

1. Boiling with water converts free gossypol to bound gossypol at an extremely rapid rate. In the preparation of Incaparina, if the mixture is boiled for as long as 10 minutes, almost all of the free gossypol will have disappeared.

Table VI
Effect of Removing Bound Gossypol on the Nutritional Value of Cottonseed Meal

Group no.	Meal description	Gossypol content Free	Gossypol content Bound	Nitrogen solubility in 0.02N NaOH	Lysine availability [2]	Chick growth [3] av. gain for 4 weeks
		%	%	%	%	g.
1	Original meal	0.033	1.32	61.0	54.9	121.7
2	Treated to remove free gossypol	.003	1.10	69.0	54.1	157.8
3	Treated to remove bound gossypol	.004	.49 [1]	77.0	70.4	222.4
4	Standard meal (butanone-extracted	.006	.37	89.0	87.5	233.2

[1] Includes 0.42% dianilino-gossypol.
[2] Three rats in each group.
[3] Twenty-five chicks in each group.
Reprinted from The Journal of the American Oil Chemist's Society, Vol XXXIV, 21. January 1957 Issue.

Table IX
Effect of Boiling Cottonseed Protein Preparations with Water

Description of Sample	Boiling Time	Free Gossypol	Total Gossypol	Bound Gossypol	Nitrogen Solubility	Lysine with Free Epsilon Amino Groups	Rat Protein Repletion Value Gain in wt. in 10 days
	Min.	*%*	*%*		*%*	*% of Protein*	*Grams*
Cottonseed Flour							
Sample No. 1	0	0.022	1.23		61	2.96	32.2
Sample No. 1	5	.015	1.27		21	3.04	34.0
Sample No. 2	0	.05	1.28				
Sample No. 2	5	.027					
Sample No. 2	15	.029					
Hexane Extracted	0	1.45	1.63	0.18	98	3.60	41.2
Cottonseed Meats	5	.12	1.67	1.55	37	3.32	
Cottonseed Meats	10	.09	1.63	1.54	36	3.15	
Cottonseed Meats	15	.07	1.62	1.55	38	3.16	35.4

2. Boiling with water does not destroy bound gossypol.
3. Boiling with water quickly reduces nitrogen solubility to a very low value. This reduction in nitrogen solubility is not paralleled by a corresponding reduction in protein quality.
4. Boiling with water does not markedly reduce the content of lysine with free epsilon amino groups unless at the same time there is conversion of free gossypol to bound gossypol.
5. Boiling with water does not reduce protein quality as determined by the rat protein repletion test unless considerable amounts of free gossypol are converted to the bound form during the boiling period.

These conclusions are tentative. Additional experiments are in progress.

DISCUSSION

Altschul: I'd like to know, Carl, whether it isn't possible that bound gossypol and epsilon amino lysine are two sides of the same coin. I would like to know if it is possible to have a meal with a high bound gossypol that is still very good, or, conversely, is it possible to have a meal with a low epsilon amino lysine but a low bound gossypol that's very good, and, thirdly, is it impossible to have a meal with a high bound gossypol and a low epsilon amino lysine? My own feeling, from looking at Vernon's and your data, is that they both measure somewhat the same thing; therefore, if you were to insist on a high epsilon amino lysine, you would almost automatically take care of the problem of bound gossypol as well.

Lyman: The question is essentially, I think, if we get good values of epsilon amino lysine, haven't we then automatically selected those meals which do have high bound gossypol? Essentially, I think this is so, although I'm sure we're going to find with extension of the work on boiling with water, some meals that have gossypol bound in another way and this would mean that total gossypol would not be a measure of what we have.

Frampton: On the question of bound gossypol, as I indicated before, this is not only gossypol that is liberated from cottonseed meal on mild acid hydrolysis, and this doesn't present that whole picture—I was in hopes that Carl would get into some other aspects of it. Take, for example, in

the case of discoloration of egg yolks that are produced by hens fed on cottonseed meals. The intensity of color and the percentage of eggs that are colored is not related to the gossypol as we measure it, free or total. This discoloration is caused by gossypol and so here we have a physiological evidence of the effect of gossypol that is quite different and quite apart from the growth response of animals.

Lyman: We have realized for years that bound gossypol is a relative matter and that we had some gossypol in between that sometimes seemed to be bound but not very firmly bound. If we try to specify exactly where bound gossypol ends and free gossypol begins, it's pretty difficult to draw a hairline there.

Curtin: Is the gossypol lost in your cooking accounted for by a corresponding decrease in epsilon amino groups?

Lyman: It can't be accounted for by decrease in epsilon amino groups. This is one experiment—we're going to have to get a lot more work. But, to me, this is a strong suggestion that there are possibilities for getting gossypol bound to something else beside lysine epsilon amino groups. It looks like boiling in water might be a way to do it.

Question: Did you measure pH in the cooking experiment?

Lyman: We did not, but typically, you are going to have a pH slightly on the acid side, 6-6.5 or so.

LIPIDS IN COTTONSEED MEAL

by

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SUMMARY

The presence of small amounts of propene ring fatty acids in cottonseed meal may exert appreciable effects on the nutritive value of cottonseed meal. It has been shown previously by Masson, *et al* [1], and by Shenstone and Vickery,[2] that these acids are implicated in the pink discoloration phenomenon of cold stored eggs from hens fed cottonseed meal. In recent experiments, *Sterculia foetida* oil which contains 40-70% of the 19 carbon propene ring sterculic acid, has been administered to laying hens to determine its influence on permeability of the vitelline membrane surrounding the yolk of eggs, on hatchability of fertile eggs, and on gossypol discoloration in the eggs. After 1 month of cold storage, a comparison of *in vitro* water uptake of yolks of eggs produced by hens fed 150 mg. *Sterculia foetida* oil with those of hens fed an equal amount of corn oil, showed 80% more water uptake by the yolks from the hens fed *Sterculia foetida* oil than from the hens fed corn oil. One hundred-fifty mg. of the oil had a marked effect on hatchability of eggs. In fertile eggs from hens fed *Sterculia foetida* oil, 80% of the embryos were dead by the 5th day of incubation, 93% by the 10th day, and 100% by the 19th day. Feeding as little as 25 mg. of the oil daily increased embryo deaths significantly. Twenty-five mg. of *Sterculia foetida* oil administred daily to hens receiving 3 or 6 mg. gossypol greatly increased gossypol discoloration in cold storage eggs. The oil caused the alkaline fluids of the white to pass more easily through the vitelline membrane into the yolk. The consequent increase in pH of the yolk caused the gossypol present to react more readily.

[1] Masson, J. C., Vavich, M. G., Heywang, B. W., and Kemmerer, A. R.; Science, 1957, v126, 751.
[2] Shenstone, F. S., Vickery, J. R.; Poultry Science, 1959, v38, 1055.

DISCUSSION

Frampton: The experiments I want to report on were carried out in cooperation with Mr. Burt Heywang of the Southwest Poultry Experiment Station, Glendale, Ariz. The meals were fed at Glendale, and the eggs that were produced were divided into two portions. One portion was shipped to New Orleans for our study, while the other portion was retained at Glendale for Mr. Heywang's observations.

The eggs were opened in New Orleans after a 6-month storage period. The pH values of the whites of the control eggs was about 9, while the pH of the yolks was about 6.7. This is in agreement with the observations made by Dr. Kemmerer for his control eggs.

Now, we have observed that the chromogen in the yolks of eggs produced by cottonseed meal-fed hens is a pH indicator, it is purple in an alkaline medium and apple green in an acidic medium. The combination of the purple of the chromogen in an alkaline medium and yellow of the xanthophylls and carotenes of the yolk gives the greenish brown color that is typical of the yolks of stored shell eggs produced from cottonseed meal. When the pH of the brown yolk is decreased to a value below pH 7, the brown color disappears. We use the change in total color of the yolk material as the pH is changed

Table I
Properties of Eggs Produced by Feeding Cottonseed Meal Fractions

Cottonseed product incorporated into laying ration	pH		Color			Halphen test
	Whites	Yolk	Whites	Intact yolk	Yolk at pH 10.4	
Control	9.0-9.1	6.5-6.8	normal	normal	normal	—
CM-108	8.0-8.1	7.9-8.5	pink	dark brown	brown	+
Residue after extraction of CM-108						
No. 1	8.8-9.1	6.5-7.3	normal	normal	brown	—
No. 2	9.0-9.1	6.4-7.0	do	do	do	—
No. 3		6.3-6.9	do	do	do	—
No. 4	6.5-6.9	6.5-6.8	do	do	do	—
No. 5		6.4-6.8	do	do	do	—
Extracts from CM-108						
No. 1	8.1-8.2	8.1-8.2	pink	translucent watery yolk apricot color		+
No. 2	9.1-9.2	6.0-6.5	normal	normal	normal	—
No. 3		6.6-7.0	do	do	do	—
No. 4		6.5-7.0	do	do	do	—
No. 5		6.3-7.2	do	do	do	—

from about 6.4 to 10.5 as a measure of the concentration of this chromogen in the yolk.

Attention is directed here to one cottonseed meal, CM-108, that was used in the swine experiment and in broiler tests, as well as in the tests with laying hens. As may be seen from the table, the eggs produced from this meal had yolks with pH values in the neighborhood of 7.9 when the eggs were opened after a 6-month storage period. The whites had pH values of about pH 8. The whites of these eggs were pink, and the yolks brown. This meal gave a positive Halphen test.

A portion of CM-108 was thoroughly extracted with hexane, and the residual meal was fed to the laying hens at a rate equivalent to the original SM-108, e.g., at a rate equivalent to 20 pounds of CM-108 per 100 pounds of total ration. I repeat, the meal was thoroughly extracted, and the residual oil was very low. The Halphen test was negative. The eggs from this meal, after 6 months of cold storage, had pH values of about 9, the yolks had pH values of about 6.5. That is, they were about the same as those noted for the control eggs. Moreover, the eggs were normal in appearance. The whites were white and the yolks yellow. When the pH of the yolk material was increased to 10.5, however, the typical brown color developed. In other words, the chromogen was present in the yolks but the typical cottonseed-yolk color did not appear because the pH of the yolks was low, but it did appear when the yolk material was made alkaline.

We combined the hexane extract with soybean meal and the mixture was fed to the laying hens at a rate equivalent to the original CM-108. This hexane extract gave a positive Halphen test. We found that the pH of the yolks, after a 6-month storage period, was about pH 8, and yolks were salmon colored and the whites were pink. The pH sensitive purple chromogen that is typical of eggs produced by cottonseed meal-fed hens was absent from these yolks since we got no color change on increasing the pH of the yolk material to pH 10.5.

Thus, it is evident that the brown color of egg yolks from eggs produced by cottonseed meal-fed hens is enhanced because of the presence in the meals of a substance that gives a positive Halphen test. When this substance is included in the diet, the pH of the whites and yolks of the eggs produced converge on storage, and with the increasing pH of the yolk (from about 6.5 to 8) there is a change in the color of the chromogen and the yolks appear brown.

The evidence is that the substance in cottonseed meal that affects the pH of the yolks of stored shell eggs also is responsible for the pink color that develops in the whites. Apparently, the permeability of the vitellin membrane is affected by this substance.

The study was extended to a number of cottonseed meals and to the eggs produced from them. We determined the intensity of the Halphen reaction for each meal, and we correlated these several data with the increase in the yolk pH and with the percentage of eggs with pink whites after the eggs had been in cold storage for 6 months. The use of the Halphen acid reaction in these correlations might be subject to some criticism since it is not a good quantitative test, as Dr. Kemmerer has pointed out. The reaction between sulfur and the cyclopropene ring is not stoichiometric, and we do not know all of the factors that affect the intensity of the color produced when the reaction is brought about in the laboratory. It is, however, the best method that is available and, for the moment, we will have to accept the errors in the determinations. Even so, we find the correlation coefficient between the intensity of the Halphen test and the pH of the yolk to be about 0.94. The correlation coefficient between the intensity of the Halphen test and the percentage of eggs with pink whites was found to be 0.91. The correlation between the intensity of the Halphen test and the percentage of oil in the cottonseed meals fed was found to be 0.91. Evidently, if all of the oil is removed from cottonseed meal going into laying rations, the incidence of pink whites and brown yolks in the stored shell eggs will be very materially reduced.

PREPARATION OF COTTONSEED PROTEIN PRODUCTS

by
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It appears that vegetable proteins have been a component of man's diet since time immemorial. Written records indicate that the soybean was a staple of Chinese food as long ago as 2838 B. C. (1)[1] However, as far as we know, it is only within recent years that the cottonseed has contributed protein for human consumption. It is reported that this use was first proposed less than a century ago (2).

As we study methods for producing protein from cottonseed, we see that several steps are involved in present processes. Almost immediately, we begin to think of variations which might be applied to these methods. Further investigation reveals that some of these have been tried with varying degrees of success. The possibilities of others, as far as we can learn, have not been explored. As we proceed with our discussion, we want to propose some of these procedures for future study, admitting that we have no concrete evidence upon which to predict their success or failure.

One method for producing cottonseed flour has been described briefly in the literature (3,4). We might look at the steps outlined in this procedure.

[1] Figures in parenthese refer to References at end of this article.

1. Seed are selected on basis of quality, an effort being made to select prime seed which are free of damaged kernels, have a low percentage of immature kernels, and are low in FFA content.
2. Cleaning and delinting are carried out in the normal manner and the delinted seed are recleaned.
3. The seed are cut in bar hullers and subjected to screening and air separation in order to remove all but traces of hull from the meats.
4. The meats are rolled to a thickness of 0.010″ or less and cooked for 70-90 minutes to a maximum temperature of 225° F.
5. Oil extraction is accomplished by pressing in hydraulic presses.
6. The cake is then aged for 30 days.
7. The aged cake is ground in hammer mills and the fine material recovered by air separation. It is stated that a considerable portion of the material is rejected during grinding and separation and a ton of seed which would yield 950 lbs. of 43% protein cake yields 300 lbs. of flour. Composition of the flour is shown in Table I.

Table I
Composition of Cottonseed Flour

Proximate Analysis	%	Mineral	%	Vitamin	Mg/g
Moisture	6.34	Phosphorus	1.26	Thiamin	10.4
Protein	57.53	Calcium	.20	Riboflavin	10.2
Fat	6.45	Magnesium	.65	Niacin	84.0
Fiber	2.06	Iron	.012	Pantothenic Acid	25.5
N-free Extract	21.38				
Ash	6.24				

At this point, an examination in more detail of the several processing steps is in order. In this discussion we intend to place special emphasis on practical aspects of operation. It should be borne in mind that, since the above information was published, there has been a large increase in the amount of cottonseed processed by continuous press and solvent extraction with a corresponding decrease in the tonnage processed through hydraulic equipment.

1. Seed Selection

Since quality of the finished product is partly dependent upon seed quality, seed of the best quality available should be selected. Oil mills usually store seed in large houses or silos. Most frequently, seed are unloaded and in storage before an analysis on them is received. Thus, it is seen that there are problems with segregation of the highest quality seed for special purposes. However, it is possible to make physical provisions for such segregation. For actual selection of seed, the logical approach would seem to be for a mill to study seed quality from the different areas of the seed buying territory. Experience gained over a period of several seasons should make it possible to obtain reasonably good selection of seed by point of origin. This selection could apply to gossypol content and ammonia content as well as to seed damage and FFA content.

2. Cleaning and Delinting

Conventional cleaning and delinting equipment is satisfactory for this operation. For the highest quality product some additional cleaning equipment is indicated. Perhaps the best location for this equipment is following the linters in order to remove any foreign material which has been loosened during delinting and handling.

3. Hulling and Separating

The standard bar huller is satisfactory here and it is desirable to open the huller for coarse hulling. This results in the production of a relatively large percentage of whole and coarse meats and a minimum of fines.

The writer believes that the highest quality product will be produced by complete purification of meats (100% hull removal). We do not say that an acceptable flour cannot be produced without this degree of purification.

Let us assume first that we will follow the route of 100% hull removal. Those familiar with oil mill operation will agree that, in this country at least, it is not economically feasible to completely purify the total meats stream. An attempt at complete purification of all meats results in excessive loss of oil to hulls. The practical procedure is to separate coarse meats from fine meats on the huller shakers and, by aspiration at the ends of the shakers, to remove a major portion of the hulls carried by the coarse meats fraction. The coarse meats are then further treated by aspiration and/or gravity tables to obtain the desired purity. The purified meats then go to extraction equipment for producing cake or meal used in making flour.

The other fractions obtained during meats purification will contain some meats and must be further treated for separation. The meats portions eventually must be combined, along with some hull, into a stream which is processed for producing cake or meal of normal protein content.

It will be realized that this process for using pure meats involves the handling of two separate streams after the hulling operation. Parallel installations of equipment for rolling, cooking, extraction and grinding are required.

As a second consideration, we can examine the possibilities of a process in which the meats are not entirely free of hulls. With adjustments in separating equipment, it is possible to raise the protein in cake or meal to 44-48% without incurring serious separation losses. You probably are aware that some mills do this at the present time, feeling that the additional capacity through extraction equipment and perhaps slight improvement in extraction justify this type of operation. Protein in meal is then reduced to the desired level by diluting with hull bran.

Regardless of the degree of purification to which meats are subjected, we recommend that the amount of lint fibers in the meats be held to a minimum. The reason for this will be apparent in later discussion. We use the term "lint fibers" here to differentiate between this material and the crude fiber contributed by hulls.

4. Rolling and Cooking

Whether or not meats are completely purified, we recommend rolling to a flake thickness of 0.010" or less. This contributes to rapid and uniform cooking by presenting a large surface and a short distance for moisture and heat penetration. A moisture content of at least 10-11% improves the rolling and helps to break some of the pigment glands at this point.

Cooking should be substantially the same on pure meats as that on meats containing some hull. We would recommend slightly more cooking on pure meats if the material is to be pressed in hydraulic equipment or given a complete pressing in continuous presses, the object being to prevent "crawling" and slippage during the pressing operation.

As in any cooking operation on cottonseed meats, we are faced with two opposing objectives. In most processes, some cooking is required to prepare the material for extraction and to reduce the free gossypol to an acceptable level. On the other side, we like the cooking operation to be as mild as possible in order to minimize damage to protein. As a compromise, we suggest starting the cook with about 13% moisture, cooking for 45-75 minutes and a finishing temperature of 215-225° F. You will realize that we are speaking in very general terms here and that variations will be required depending on the type of extraction which is to follow. The moisture content of the cooked material must be adjusted to the optimum for the extraction process and in the case of continuous presses somewhat higher drying temperatures are required.

5. Oil Extraction

We have a number of choices here which include hydraulic pressing, continuous press operation, filtration extraction, prepress-solvent extraction and direct solvent extraction. Any of these should produce an acceptable product. With direct extraction, which employs little or no cooking, gossypol presents a problem. It is necessary that gossypol be reduced to acceptable levels and the use of special solvents would seem to provide the logical answer. The lower oil content obtained with the solvent processes should contribute to better keeping quality. The drastic treatment associated with continuous press operation can be expected to lower protein solubility.

Without attempting any detail, we would like to mention the extraction with mixed solvents which was recently developed in this laboratory by Dr. Frampton and Mr. King. This process which employs little heat for protein damage and which removes most of the gossypol during extraction offers interesting possibilities and I am sure we will hear more about it during this meeting.

6. Aging Cake

Certainly, the cake should be held for several days to allow thorough cooling before grinding. Whether or not further aging is desirable, the writer is not prepared to say.

7. Grinding and Separating

The usual choice for grinding is the hammer mill. Also, heavy impact type hammers seem preferable to thin hammers which give some cutting action as well as impact.

It has occured to us that an attrition mill might be preferred if a process is used in which meats are not entirely free of hulls. We have no data to substantiate such a choice but the reasoning is that hulls tend to flatten and pass between the plates of an attrition mill. Therefore, the attrition mill might produce less fine material of a nonprotein nature than would be the case with a hammer mill.

Air flotation is a practical method for separating the fine flour from coarser and heavier materials and it appears that this procedure has been used on most of the flour produced in this country. It is recommended that equipment be operated on open cycle, that is, material rejected in the air separation should be diverted to other uses and not recycled to the grinder. However, where pure meats are processed, it does appear that a major portion of the material can be recovered as flour without any serious reduction in protein content.

Previously, we have mentioned the desirability of having meats free of lint fibers. One reason for this is that these fibers are airborne and concentrate in the light flour fraction. A striking example of this was obtained in a series of tests on a normal prepress-solvent extracted meal of about 43% protein. With such a meal it might be expected that the protein content of recovered flour would decrease as

the percentage of recovery is increased. However, the protein actually increased from 47% to nearly 50% as the percentage of recovery was increased from 4% to 40%. We interpret this to mean that the lint fibers are among the first material to be airborne. Thus, most of the fibers concentrate in the light fraction and with a low yield of this fraction the fibers have a greater diluting effect on the protein present. Unfortunately, at the time these tests were made, we did not attach too much significance to this behavior and we failed to obtain crude fiber analyses on the samples.

It has been said (3) that bolting of the flour through silk has not been found practicable. However, this statement was made concerning material pressed in hydraulic equipment and which probably contained about 6% oil. It is entirely possible that solvent extracted material containing 1% or less of oil could be bolted through silk. We have not tried this but it seems worth investigating.

Sifting through a fine mesh screen is another method of separating which might produce an acceptable product. Again, we would like to emphasize the importance of having the starting material reasonably free of lint fibers. Those who have seen meal, or even hull pepper from lint beaters, passed over a shaker clothed with fine mesh screen and watched the formation of little fiber balls caused by the vibrating action might guess that this would give efficient separation of fibers. However, such is not the case. If meal containing fibers is sifted through 100-mesh screen and the resulting product examined under the microscope, it is quite surprising to see the amount of fibers passing through the screen.

Solvent extraction operations, including prepressing, do not imbed hull particles into protein material as tightly as in the case for hydraulic and screw press operations. This should allow reducing the protein bearing material to the desired fineness with less reduction in hull particle size and facilitate separation of the hull.

We believe the data in Table II give hope that an acceptable product can be produced by screening.

The original meal was a prepress-solvent extracted meal. No elaborate purifying equipment was available at this mill. Coarse meats were scalped heavily at the ends of huller shakers and sent to the process. All other fractions, including fine meats, tailings, beater fiber, etc., were held out of the process stream while producing this special meal. You will realize, from the protein and fiber contents, that meats going to the process contained a significant amount of hulls.

Table II

Sample Description	Moist.	Oil	Prot.	Crude Fiber	Flour Yield
Original meal	6.4	1.68	45.31	8.20	—
Ground in lab. attrition mill, sifted through 100-mesh screen on SWECO 18″ test separator. Feed rate 200 lb./hr.	8.3	1.75	51.20	3.7	16%
Ground in Raymond No. 10 IMP mill with impact hammers, separated in Raymond 30″ Whizzer	6.8	2.50	50.75	—	24%
Ground in Raymond No. 10 IMP mill with impact hammers, separated in Raymond 30″ Whizzer	6.8	2.38	51.12	4.8	32%

Recent Work

It was suggested that we include here some results and discussion of recent tests on preparation of cottonseed flour. These tests were made under the auspices of Mr. Layton E. Allen of UNICEF who arranged for the necessary equipment. Southland Cotton Oil Co. of Waxahachie, Texas, provided space, installed the equipment and furnished help for conducting the tests. Gen. Patton and Dr. Frampton of this laboratory joined us for the tests, did the work of collecting the required samples, and arranged for analyses on the samples.

Several mills in Mexico and Central America furnished raw materials in the form of cottonseed cake or meal. The raw materials were ground in a No. 10 Raymond IMP mill using two different types of hammers. The ground materials were then separated into fine and coarse fractions using a Raymond 30″ Whizzer. Three speeds were employed with the Whizzer in order to vary the percentage of fines recovered. This resulted in a large mass of data and no attempt will be made at complete coverage here.

Table III gives a brief summary of representative data. All samples in this table were ground with heavy impact type hammers and separated with a Whizzer speed of 1200 R.P.M.

The nitrogen and fiber contents of all these raw materials indicate that they were not produced from pure meats but rather from meats containing some hulls.

The fines fractions are higher in nitrogen and lower in crude fiber than the starting ma-

Table III

Mill	Process	Sample	% Yield	% Oil	Dry Basis % Gossypol Total	% Gossypol Free	% Crude Fiber	% Nitrogen	Lysine G/16 g. of N
A	Prepress-Sol.	Original	—	1.80	1.52	0.087	9.22	7.85	2.97
		Ground	—	1.96	1.55	.086	8.82	7.94	3.03
		Fines	32	2.55	1.76	.101	5.13	8.78	3.35
		Tailings	68	1.66	1.40	.077	11.63	7.31	3.31
A	Prepress-Sol.	Original	—	.62	1.31	.050	16.00	6.64	2.69
		Ground	—	.68	1.29	.049	16.16	6.47	2.73
		Fines	20	1.23	1.68	.065	9.20	8.11	2.90
		Tailings	80	.58	1.31	.043	17.30	6.15	3.07
B	Prepress-Sol.	Original	—	2.19	.96	.046	7.65	8.16	3.53
		Ground	—	2.23	.98	.043	8.37	8.48	3.32
		Fines	25	2.93	1.08	.057	3.82	9.46	3.59
		Tailings	75	1.99	.96	.043	9.30	8.28	3.19
C	Screw Press	Original	—	5.87	.93	.047	9.46	7.89	3.31
		Ground	—	5.68	.90	.044	10.68	7.64	3.19
		Fines	23	7.94	1.04	.057	4.53	8.68	3.50
		Tailings	77	5.35	.85	.043	10.78	7.60	3.28
D	Screw Press	Original	—	5.79	1.32	.046	10.69	7.68	2.99
		Ground	—	5.60	1.25	.047	11.29	7.41	3.08
		Fines	30	7.09	1.42	.057	6.98	8.36	3.13
		Tailings	70	5.15	1.22	.045	12.29	7.12	3.18

terial. However, this air flotation does not give a clean cut separation of crude fiber. If the highest possible protein and the lowest possible crude fiber are desired, it is necessary to start with a raw material produced from pure meats.

Two samples from Mill A were included. This was done to show the effects of wide variation in nitrogen and crude fiber contents of the raw material. The raw material containing 7.85% nitrogen produced 32% of fines containing 8.78% nitrogen while the raw material with 6.64% nitrogen produced only 20% fines with a nitrogen content of 8.11%.

It will be noted that considerable variation exists in the gossypol contents of the several raw materials. Some of this, we expect, is due to variations in processing conditions. However, a part probably is due to variations in gossypol contents of the original seed.

A very important point is that there was no reduction in lysine by the grinding and separation steps. Some heat was generated during grinding but it appears that conditions were not severe enough to cause destruction of lysine.

General Discussion

Our discussion has been confined to the production of a flour type product. There is the possibility of producing protein isolates and other products which are intermediate in protein content between the isolates and the flours. However, the isolates are much more expensive than the flours. Also, we have present those who are far better qualified than the writer for discussing isolates. If there is interest in products of this type, we would prefer that information be developed during the discussion period which is to follow.

Finally, let us look at what we believe to be desirable characteristics of the finished product. At this point, we will not attempt

to set any actual limits for chemical analyses, etc. It is our understanding that this is to come up for discussion later in the meeting.

We believe the product should be relatively bland in flavor. Certainly, there should be no strong, disagreeable flavor or odor.

In certain instances, some fat in the product may be desirable from a nutritional standpoint. In general, we would favor a low fat content for better keeping quality.

Since protein is our prime consideration here, a high protein content would seem desirable. Also, we want high quality protein which has not been damaged by excessive heat treatments.

Gossypol content, both free and total, should be as low as possible.

As just mentioned, a high protein content seems desirable. Since crude fiber dilutes the protein, it follows that the fiber content should be low. The writer does not know the effects or the importance of fiber in human nutrition. We believe this question will have to be answered by Dr. Scrimshaw and others engaged in nutrition studies.

And last, but not least, we want a product that has been produced in a plant where sanitary conditions meet the requirements and standards set for the processing of foods for human consumption.

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DISCUSSION

Meinke: Is gossypol content of seed associated with variety or environment?

Fincher: Some years ago, we collected samples from East of the Mississippi all the way over into the California area. We found that any variety would tend to maintain its characteristics, whether high or low, regardless of the area in which it's planted; so that is a variety difference. That certainly is one of the differences, but also, we found that there are variations in the area in which you plant—whether your seed are grown in an irrigated area or otherwise—both of those factors do play a part.

Hopper: What is the problem? Why not get higher yields of the protein concentrate?

Fincher: I think, and I believe I indicated in the paper, you can get higher yields—I'm sure you can—provided you purify your meats in the beginning of the process. We can get higher yields here, too—we can go up to maybe 40%—but as you go higher than that, if you are working with a material that contains some hull, then you begin to get the hull particles over into your finished product. I think that's the only problem.

Question: Can you reduce the size of the hulls by grinding?

Fincher: We can reduce them, but we don't want them in the product because they decrease the protein content and increase the crude fiber in the product. The material that we are separating—the one that we want to recover—does not have the hull in it. Bear this in mind, that if we have a 25% yield of flour here, we are not throwing away that 75%, that is a good animal feed—that would go into mixed feed. I think you can recover the major portion of the cake as flour provided you

will start with a material free of hulls, but that process I think is going to be so much more expensive. I would say if we could take one of our mills in Mexico and we want to produce this product, if we could recover 5% we would be very happy. That's all we would need—that would be more, I think, than we would need. The other material is not a loss, of course, it goes into feed.

Question: Is the hull the main portion of the fiber?

Fincher: Yes. There is a little bit of lint fiber, but the hull is the main portion.

Question: To what extent would you say the proportion of crude fiber is hull particles?

Fincher: I would—this is a pure guess—I would say 85-90% of the crude fiber is in the form of hull particle rather than fine fibers.

Question: What is the basis for stopping at a yield of 25%?

Fincher: That was taken from the literature and the reason given at that time, and I think it's still valid, is that if you have some hull in your starting material and you want to make the highest quality product, if you recycle you will eventually begin to grind up those hull particles —the stuff that's rejected in the first preparation.

Lyman: Would you improve the hull separation if the lint were not removed?

Fincher: Correct, Dr. Lyman. You do introduce one other problem there though. The hulling operation without some lint removed is a little bit difficult. In cutting the seed you tend to mash some of them and increase your separation loss, that is, the oil content of the hull. But you are correct; it is much easier to remove those hull particles that have some fibers on them. It's not an insurmountable problem, but you would probably need some more hulling and separating machinery because, if you have very much lint on the hulls, the bed on the shakers becomes rather deep. You not only increase your absorbed oil loss but tend to pass some of the meats over with those hulls— you don't shake them out thoroughly. But it's not an insurmountable problem, it's something that can be done; in fact, we did it one year in Mexico at some of our mills when we reached the point where it didn't pay to cut the lint and we quit cutting it.

Fincher: Mr. Patton just mentioned that since the residue there does go to a feed material, you don't have a great deal of loss and certainly I think right now it is more economical to reduce your yield of flour and throw the other material into the feed than it is to try to get a complete separation of the hull and then recover more of the cake as flour.

Scrimshaw: I will try to put a partial answer in the record on this matter of crude fiber. Of course, there is no single answer because it depends on the proportion of cottonseed flour in a mixture and what it's going to be mixed with. INCAP mixture 8 had 3.2% of crude fiber; part of this came from sesame, a little from corn, and the rest from cottonseed flour. There was no difficulty giving this even to severely ill children. The UN Protein Advisory Group, in its general standards, mentioned 2-½%. We don't see any reason to insist on this 2-½% except as part of a general effort to get a better cottonseed flour and because if the crude fiber goes up, essential factors are going to come down proportionately. But in terms of human nutrition, it isn't worth straining to get out that last bit of crude fiber if it isn't economically convenient to do so.

Question: How does the ammonia or the protein content of seeds vary?

Fincher: In some areas, I believe, 4 or a little better percent ammonia is normal for cottonseed. We have seen seed down as low as 3-½% ammonia, which is getting down pretty low; you can hardly make a 41% protein meal out of such seeds; so that is a problem in some places.

Question: Where the seed is low in protein, what other constituent increases?

Fincher: In general, you will find a pattern that the oil increases as the protein decreases. Also, you will find some seed that will be higher in hull content than others; that affects the ammonia and the oil content of the seed. Let's talk about meats just a little bit. You find a pretty general pattern that there is an increase in oil as the protein decreases. Whether or not there is a change in other constituents such as carbohydrates, I don't know.

AZEOTROPE EXTRACTED COTTONSEED MEALS

by
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Advances in the improvement of the nutritive quality of cottonseed meal have been comparatively recent, considering that cottonseed has been processed in the United States for nearly 100 years. Moist cooking prior to hydraulic pressing produces meals satisfactory for ruminants. Control of condition of screw pressing and prepress-solvent extraction has made it possible to obtain meals useful in broiler rations, in some cases usable as a portion of the protein supplement in rations for laying hens producing eggs for the fresh egg market. The objective of more recent investigations has been to provide meals satisfactory for feeding without restriction to all classes of livestock, including laying hens and swine. Hence, attention has been given to the removal or inactivation of gossypol without reduction in available lysine. The most recent approach at the Southern Utilization Research and Development Division has been the extraction of the cottonseed meats with an acetone-hexane-water solvent mixture by King, Kuck, and Frampton (1, 2).

The object has been to rupture the pigment glands containing gossypol and to extract the gossypol without it reacting with lysine. Electron microscopic examination of the glands of seed of the Deltapine variety of cotton by Moore and Rollins (3) demonstrated that glands have a complex internal structure in which discrete particles of gossypol ranging in size from .1 micron to less than 0.2 micron in diameter are held within a membraneous meshlike network. These particles exhibit no birefringence in the gland or when extruded. Calculations of specific surface based on sizes of the particles and density of purified gossypol indicate a surface area per gram of the order of 8 square meters.

Cottonseed are variable in composition owing to differences in environment and variety of growth. Consequently, meals and flours obtained by currently used processes are variable in composition and nutritive value. Variations in oil, nitrogen, and gossypol contents of cottonseed kernels found in a symmetrically designed investigation, involving the growing of 8 varieties at 13 locations during three growing seasons are listed in Table I (5). Particular attention is called to the wide variation in the nitrogen-gossypol ration. It would seem that meals having reduced available lysine would result from the use of most of the processing methods used currently unless seed having a high nitrogen-gossypol are selected.

Table I

Variation in Composition of Cottonseed Kernels

	Oil	Nitrogen	Gossypol	Nitrogen Gossypol ratio
	%	%	%	
Moisture-free kernels				
Low	26.8	4.75	—0.39	
High	43.4	7.34	1.70	
Mean	36.4	6.31	1.14	
S. D.	2.98	.52	.26	
Moisture- and oil-free kernels				
Low		8.38	0.57	2.93
High		10.99	2.98	18.15
Mean		9.90	1.80	6.00
S. D.		.53	.46	2.15

N = 312

Relationships have been determined which demonstrate that the gossypol content of cottonseed kernels are positively and highly significantly correlated with the oil content and that both gossypol and oil contents are negatively and highly significantly correlated with nitrogen content (Table II) (5). The gossypol and oil contents are positively and significantly

correlated with the inches of rainfall occurring during the maturation period of the growth and development of cottonseed (4, 6).

Table II

Relations Between Specified Cottonseed Constituents

	Correlation coefficient
Gossypol vs. oil	0.65 to 0.71
Gossypol vs. nitrogen	— .59 to — .76
Oil vs. nitrogen	— .66 to — .88

8 varieties N for each = 39

Recognizing that lysine is heat labile, the adverse effects of gossypol, and the variations in the composition of cottonseed, search has been made for improved features of practical methods of processing cottonseed to obtain meals of highest nutritive quality for nunruminants. This has resulted in finding that a superior quality meal can be obtained by use of a mixture of acetone, commercial hexane, and water in the proportion of 53, 44 and 3 parts by volume, respectively, for extraction of the oil. The solvent recovery should pose no impossible problems since it forms a constant boiling mixture, 48°-52° C. (118°-125° F.). In the laboratory experiments, cottonseed meats were moistened and equilibrated to 10-15% water content, flaked to 0.003", dried to about 10% moisture, and extracted by batch and continuous methods with the mixed solvent. There seemed to be no trouble on account of fines. The efficiency of the mixed solvent for extraction of oil and gossypol was determined in comparison with those of acetone, hexane, and water. It proved to be the most efficient (Figures 1 and 2), particularly for the extraction of unbound gossypol (1).

Experience to date indicates that by the use of the mixed solvent (or azeotrope) the meals obtainable contain almost no free gossypol, a low level of total gossypol, and a low residual oil content without loss of epsilon-amino-free lysine (available lysine) and should have excellent nutritional qualities (Table III).

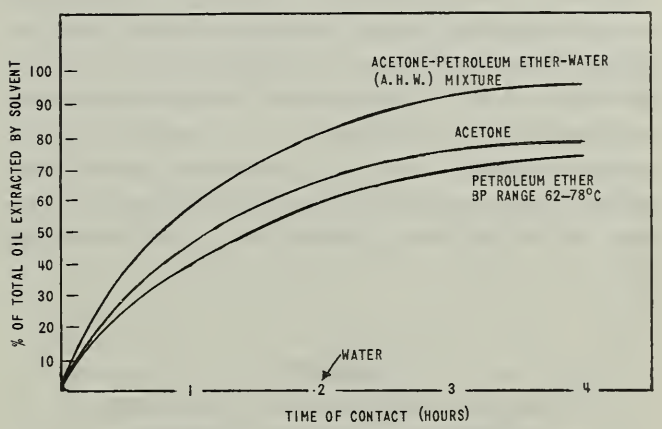

Fig. 1. Relative efficiency of specified solvents for extracting oil from cottonseed flakes.

Table III

Chemical Data for Specified Cottonseed Meals

Constituent	Commercial meals				Experimental "acetone-petroleum ether-water" extracted		Raw meats
	Prepress solvent extracted	Screw pressed	Solvent extracted	Hydraulic pressed	Batch extracted	Continuous extraction	
Free gossypol (%)	0.06	0.03	0.20	0.10	0.03	0.00	1.0
Total gossypol (%)	1.3	1.3	1.0	1.2	.25	.40	1.0
Epsilon-amino-free lysine (g./16g. N)	3.7	3.1	3.8	3.4	4.3	4.3	4.2
Oil (%)	1.0	2.5	.8	5.0	.4	.1	33.0

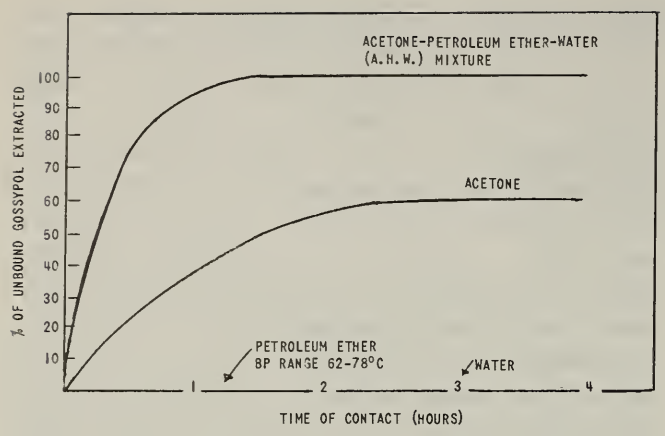

Fig. 2. Relative efficiency of specified solvents for extraction of unbound gossypol from cottonseed flakes.

In the preparation of 15-pound quantities of azeotrope extracted meals for chick feeding tests, some conditions of batch extraction of the meats of a single lot of cottonseed were varied-slight variations in composition of the solvent mixture, time of contact of meats with solvent, and completeness of extraction. The data on eight lots of meal are given in Table IV.

Table IV
Composition Properties of Azeotrope Extracted Cottonseed Meals, Extraction Conditions Varied

Meal	Oil	N_2	Gossypol Free	Gossypol Bound	Gossypol Total	Epsilon-amino lysine
	%	%	%	%	%	*g./16g. N*
1	0.58	10.01	0.03	0.34	0.37	3.98
2	.30	10.05	.05	.44	.49	4.11
3	.16	10.33	.03	.31	.34	4.22
4	2.70	9.79	.06	.56	.62	3.86
5	.67	10.12	.05	.34	.39	3.98
8	1.00	10.00	.31	.40	.71	4.32
9	1.02	10.03	.16	.46	.62	4.36
10	.77	10.12	.08	.41	.49	3.98

Two tons of the meal prepared by extraction of the meats of Acala 4-42 cottonseed with the azeotrope have been used in poultry and swine nutritional tests.

Though the detailed results have not been analyzed and published, some indications of the nutritive value of the azeotrope extracted meal can be offered. These are:

1. It has a good potential for use as a protein supplement in swine rations.
2. It should be satisfactory for use in broiler rations.
3. It is very promising for use in rations for laying hens providing the residual oil content is low. The chromophore contributing to the development of off-color in stored shell eggs appears to be present in low quantities. The pH of the yolk of stored shell eggs laid by hens fed the experimental meals appears to be normal.
4. If the oil content of the meal is low, there should be little or no pink color develop in the whites of stored shell eggs.

The promise of the results of the feeding tests conducted to date by nutritional cooperators has warranted the Engineering and Development Laboratory undertaking development research on the use of the acetone-hexane-water mixture for commercial processing of cottonseed for oil and meal. It is recognized that there are practical problems involved. The engineers have approached the problem objectively. There is a basis for strong hopes. Enough water is present to rupture the pigment glands. No appreciable heating is needed until desolventization step. The residual oil can be reduced to a low level in the meal. It is apparent that aside from any reduction owing to reaction with sugars, the availability of lysine can be preserved.

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DISCUSSION

Patton (Chairman): The azeotrope extraction appeared to be so popular that we put some engineers on that problem. Mr. Gastrock is in charge of that work. It may answer some of your questions if he will outline briefly what he is doing to get this into the pilot plant stage.

Gastrock: As Mr. Hopper told you, this azeotropic extraction process undertakes to do the following: extract with the oil essentially all of the gossypol—that means practically to remove about 70-80% of the gossypol, the remnant being mostly inadvertently bound gossypol and with less than about 0.04% of free gossypol present. It also undertakes to avoid the destruction of the fine epsilon amino lysine content which may result from combination of lysine with gossypol or sugars, heat damage, or other causes. It also undertakes to reduce the lipid content to perhaps lower values than any of us here have been accustomed to seeing, mainly for the purpose of reducing the "Halphen acid" present. That figure has been indicated as perhaps as low as 1% residual lipids. If the hexane-acetone- water extraction process is to achieve commercial status, it must be obvious that the advantages, efficiencies, and economics of the new process must be comparable to existing processes; also, there should be no outstanding disadvantages, and it is preferred that the new process be operable in existing plants with minimum modification. We have, as you see, a process which undertakes to make an unusual—a not usually encountered product—by not usually encountered methods; so we will probably have to approach that problem by unusual engineering methods. I'm not saying that this is going to be an impossible problem, but I just want to point out that it is not a simple or easy problem.

We will undertake to investigate the various types—that is, from a preliminary basis—of extractors to determine in our own mind which type of extraction process might be best suitable. If we find none, we may have to devise a new extraction process. The other problems involved are those involved in the moisture content of the extracting solvents. Please remember that moisture is one of the things that is purposely avoided in any solvent extraction process. We are purposely introducing water. We are doing it for a purpose but we must remind you that we left it out for a purpose in the existing plants; so we will study the optimum moisture content—the effect of these moisture contents on the extraction rate and on the operation of the process. The processing temperatures have been indicated as room temperature. Well, if those are the limits, we will have to abide by them and we will see what effect these have on the other conditions. The temperatures for desolventization of the oil and meal—we don't consider those as presenting any engineering difficulties; it means that we may have to use vacuum and the processes that are involved will, of course, require some slight design changes in the equipment. The oil quality we think also can be handled satisfactorily because it has been shown that prompt refining can probably take care of the gossypol, but I just wanted to point out that we are aware of the problems and we are going to try to solve them one by one and we hope that perhaps—I wouldn't want to say any date—in the future we will be able to make a progress report on our work.

Frampton: The real problem in connection with this thing is that of preparing meats in such a way that the integrity is retained and one doesn't get fines produced. I am passing out here a bunch of meats that were prepared in the pilot plant; these are typical and you can see that there are no fines and the fines problem will be reduced considerably. We have a sample of refined and refined and bleached oil—these also prepared in the pilot plant in the regular process that we had been using. The nutritive quality of this particular meal will be just about as good as we can get. The stored eggs produced from these meals are essentially normal, there is a trace of color in the eggs when the pH of the yolks is raised. The brown color of the yolks apparently can be handled with using this meal.

AIR CLASSIFICATION AND CHLORINE TREATMENT PROCESS FOR THE PRODUCTION OF LOW GOSSYPOL CONTENT COTTONSEED FLOURS[1]

by
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The laboratory approach to the problem of the production of low gossypol content cottonseed flour has been from two diverse directions; namely,

1. Air classification as a means of removing fine meal particles from intact gossypol containing glands and,
2. Utilization of chlorine as a chemical agent in the production of low gossypol content cottonseed flours or meals.

Air Classification Studies

The primary consideration for the successful production of low gossypol content cottonseed meals by air classification is to employ processing conditions which do not cause excessive rupture or fragmentation of the gossypol glands present in the starting raw cottonseed kernels. That is, disintegration of the raw seed prior to solvent extraction, moisture content control of the raw kernels, oil extraction procedure, desolventizing and subsequent pulverization of the desolventized meal all should be such as to produce minimum rupture of the glands. This criteria is in keeping with the conditions imposed upon the Flotation Process reported[2] and patented[3] by workers here at the Southern Regional Research Laboratory.

Results of preliminary studies in the Chemurgic Research Laboratory, a division of the Texas Engineering Experiment Station of the A. & M. College of Texas, indicate the following conditions are desirable for a successful air classification process:

1. Whole seed kernels or large particles of kernels should be employed. It is not essential that this kernel product be devoid of hulls because they are readily removed in the residue of the air classification process.
2. Moisture content of the kernels prior to disintegration should range from 4 to 6 percent moisture. Data to date have not established this range as a definite limitation and may be subject to change by subsequent investigations in the laboratory.
3. Solvents employed for oil extraction should be nonpolar which do not cause rupture of the glands; that is, hexane and similar hydrocarbons or the chlorinated hydrocarbons. Again this preliminary restriction on solvent may be removed when the concept of chlorine treatment is considered.
4. Process for extraction of oil from the disintegrated kernel fines in laboratory studies has involved batch extraction with hexane at room temperature. Hexane was added to kernel fines with agitation. The slurry was allowed to settle and the supernatant liquid and wet fines were freed of excess solvent-oil miscella by filtration. The process of addition of hexane, settling and filtration was repeated to remove excess oil. In general, the oil contents of meals were below 5 percent.

Yet to be considered in the air classification process is the slurry comminution of pre-extracted flakes employed by the workers here at the Southern Regional Research Laboratory. This procedure involves rolling of the kernels with adequate moisture to produce flakes and then extracting with hexane in a manner common to commercial hexane plants. Moist hexane flakes are then disintegrated as a slurry in hexane. A variation to this procedure involves rolling of seed, moisture reduction of rolled flakes to approximately 4 percent and then slurry disintegration in solvent.

[1] This paper is based on a research project being conducted cooperatively with the Cotton Research Committee of Texas.
[2] Pilot-Plant Fractionation of Cottonseed—Oil Mill Gazetteer, 54, 11-17 (September 1949).
[3] Process for the Fractionation of Cottonseed—U. S. Patent 2,579,526 (July 20, 1948).

The first experiment on air classification was made on hexane extracted meal prepared from kernel fines disintegrated in an "attrition" type mill—a type of mill in which disintegration of the kernels was realized by impacts between the kernels themselves or by impact of the kernels with the walls of the mill. Air classification was carried out in a flask which was connected to 7 one-foot sections of 2-inch plastic tubing by an adaptor. End section of the tube was covered with a fabric bag to remove fines. Air from compressed air line was blown into the flask. Particles entrained in the air thus passed into the tube and settled out in the different sections of the tube. Free gossypol and protein values on samples collected from the component parts of the classifier system (flask, adaptor, sections of plastic tube, and fabric bag) are presented in Table 1. No quantitative yield of the different fractions was made nor was an effort exerted to remove all fines from the flask. The data of Table 1 demonstrate an increasing protein content of cottonseed flour fines with increasing distance removed from the flask. For example, 53.6 percent protein in the first section of tube removed from the flask and 62.4 percent protein for a composite sample of the last 3 sections and the fiber bag. Free gossypol values, however, decreased with distance or sections removed from the flask; that is, 0.124 percent for the first section of tube and 0.088 percent for the composite of the last three sections of the tube and bag. Microscopic examinations of the fines in the tube also indicated a decrease in intact pigment glands or gland fragments as the distance from the flask increased. On the other hand, intact pigment glands were readily visible in large numbers in both the original meal and the flask residue.

Table I
Free Gossypol and Protein Values of Air Classified Cottonseed Flour Fractions

	Free Gossypol %	*Protein* %
Original Meal	1.080	50.6
Flask Residue	1.188	50.5
First Section of tube	.124	53.6
Second Section of tube	.120	56.6
Third Section of tube	.080	(52.9?)
Fourth Section of tube	.092	61.5
Final 3 Sections plus bag	.088	62.4

These data of the first air classification study indicate two facts. One, the partition type mill is capable of producing raw kernel fines without excessive rupture of pigment glands and, two, essentially gland-free cottonseed flour can be achieved by air classification. Rolling as a means of disintegrating raw cottonseed kernels was next investigated. For this Study, 5-high rolls common to cottonseed processing mills were employed. Three batches of seed were taken from the same large batch and fed into the 5-high rolls such that the three different batches of seed each received a different degree of rolling; namely, 1 pass between 2 rolls, 2 passes between 3 rolls and 4 passes between 5 rolls. The rolled meats were then batch extracted with hexane at room temperature and air dried. Free gossypol and volatile matter are indicated in Table II for the three meals and the first two fractions (approximately 30 percent of the weight of the meal supplied to the classifier) obtained by air classification.

Table II
Effect of Rolling on Free Gossypol Content of Air Classified Cottonseed Flours

No. of Passes Between Rolls	Free Gossypol, Percent		
	Original Meal	First Air Classified Fraction	Second Air Classified Fraction
1	1.130	0.212	0.200
2	1.070	.312	.444
4	.904	.472	.432

Moisture content of raw kernels—6.02.
Moisture content of original meals—6.26, 5.90 and 6.57 for 1, 2, and 4 passes, respectively.

The two air classified fractions of Table II were obtained as follows:

> Samples of meal were placed on a fine wire mesh in the cone section of the air classifier—Figure I. Cone section was then connected to the 4-foot straight section of the classifier having a 90° ell fixed at the top. Exit end of the ell was fitted with a fabric bag for collection of the meal fines. Air was supplied at the bottom of the cone beneath the fine screen wire. Air thus passed through the meal sample and carried meal fines up into the fabric bag. Air was shut off after 10-15 minutes and the sample collect-

ed from the fabric bag. Residue on the screen was removed, placed in a mortar, mulled with a pestle and returned to the air classifier for collection of the second fraction listed in Table II.

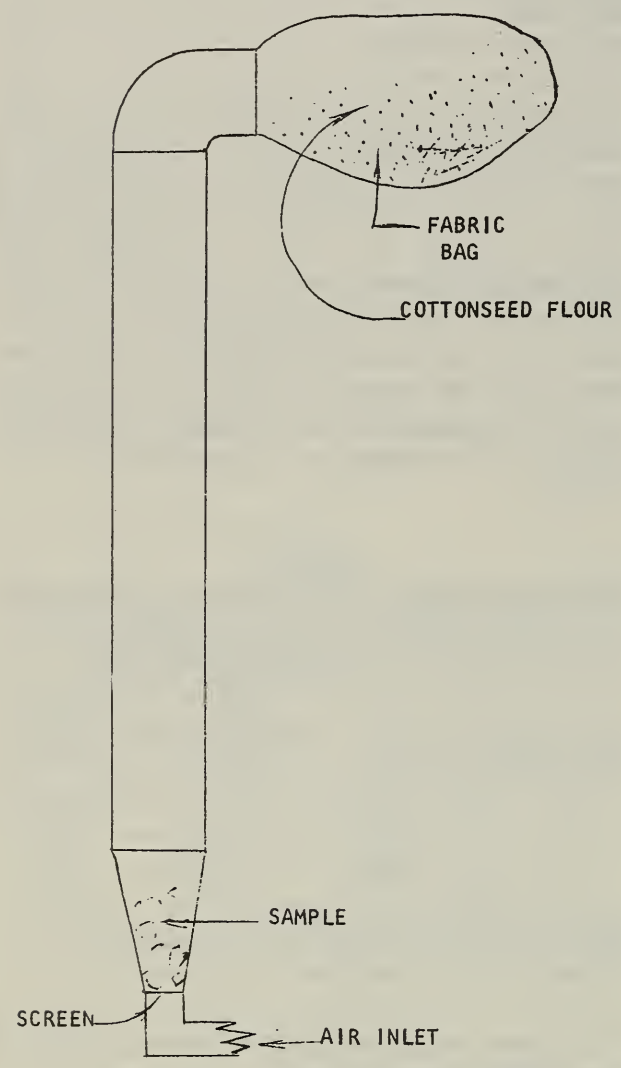

FIGURE 1

Data of Table II indicate a greater release of free gossypol or gland fragmentation as more mechanical work is done on the kernels —mechanical work being expressed as number of passes between rolls. Microscopic examination of the fractions revealed the presence of gland fragments in the 2 and 4 pass air classified fractions but essentially no gland fragments or intact glands in the air classified fractions obtained from the 1 pass meal.

The next question critical to the successful production of cottonseed flour by air classification is the yield that can be obtained. Rolling of raw kernels through single pass rolls indicates a yield of approximately 30 percent of flour fines based on hexane extracted meal put to the classifier. Meal fines, 2 to 40 microns in size, amounting to 70 percent of the starting meal have been reported—for the slurry comminution technique of the Southern Regional Laboratory workers. In this respect, the slurry grinding would be desirable provided subsequent air classification of meals prepared by this technique is possible. Another means of upping the yield of air classified flour would be to further disintegrate the extracted meal prior to air classification. It is true that the glands of the extracted meal are more friable than the glands present in the full oil kernel meats. Yet, the data of Table III show the feasibility of applying limited grinding or mulling energy to the extracted meal.

Table III
Yield of Cottonseed Flour Obtained by Air Classification of Hexane Extracted Cottonseed Meal

Fraction	Yield, Percent	Free Gossypol, Percent
1	18.5	0.078
2	10.5	.084
3	9.0	.124
4	11.5	.124
5	17.5	(.368)?
6	5.0	.230
7	24.0	3.40
8	4	.630

Results obtained with 200 gram sample of meal.

Data in Table III were obtained with cottonseed kernels containing approximately 4 percent moisture. These kernels were put through 5-high rolls and then extracted with hexane. The meal was subjected to air classification and the flour collected in the fabric bag. Residue meal in the classifier was removed and mulled in a mortar with a pestle and again returned to the air classifier. This procedure of air classification and mulling of the residue was continued until 6 flour fractions, a gland-meal residue and a coarse-meal residue were obtained. The gland-meal residue (fraction 7 of Table III) was collected in the fabric bag

by increasing the air flow to the classifier. A composite sample of the first four fractions indicated in Table III would contain less than 0.1 percent free gossypol and would comprise 48.5 percent of the starting hexane extracted meal put into the air classifier. A combination of the first six fractions would represent a yield of 72 percent and a free gossypol content of approximately 0.18 percent as compared to a free gossypol content in the initial hexane meal of 0.960 percent free gossypol. These data support the premise that mechanical work can be done on both the raw kernels and the hexane extracted meal without causing excessive rupture of the pigment glands and thereby producing a yield of 50 percent cottonseed flour of less than 0.10 percent free gossypol.

Theoretical reason for the separation of the intact glands from meal fines is, in all probability, based on shape difference rather than density per se. That is, the ovoid or spherical glands are more difficult to suspend in an air stream than the angular meal fines or it may be said there is less drag on the ovoid gland particles by the air stream than on the angular meal particles. This concept is in line with the results obtained in the solvent flotation technique developed by the Southern Regional Laboratory workers who reported a greater settling rate of the glands as compared to meal fines when both were suspended in hexane or hexane and oil medium. This faster rate of settling of the glands is realized despite the lower density of the glands as compared to the meal fines—1.26 to 1.38 grams per cc for the glands as compared to 1.40 to 1.45 grams per cc for the meal tissue. In air classification, the meal tissue with the higher density is removed from the glands of lower density.

Chlorine Study

Although the air classified cottonseed flour preparations mentioned in the earlier parts of this presentation are low in free gossypol, a means of further reducing the free and total gossypol values would be desirable. It is evident that the free gossypol released from the glands to the meal by processing procedures or free gossypol present outside of the glands in the starting kernels cannot be removed by air classification. Two approaches to the problem present themselves:

1. Extraction of air classified meal with a polar solvent and
2. Chemical treatment.

Some representative results obtained by extraction of a series of air classified samples with 80 percent aqueous isopropanol are indicated in Table IV. Free gossypol values are reduced by this extraction procedure and protein values are increased (by extraction of nonprotein components). However, appreciable total gossypol remains in the extracted samples. The effect of chlorine on both the free and total gossypol contents of an air classified flour sample, representing a 50 percent yield of flour based on starting meal, is demonstrated by the data of Table V. Composite air classified samples contained 0.072 and 0.312 percent free and total gossypol, respectively, prior to chlorine action as compared to values of 0.020 and 0.028 percent free and total gossypol after treatment with chlorine. The chlorine action was accomplished in a slurry of the air classified meal in hexane. Chlorine gas, generated from manganese dioxide and hydrochloric acid, was bubbled through sulfuric acid and then into the hexane slurry of the classified flour. Solvent was removed from the meal fines by filtration and the moist fines air dried at room temperature followed by a 30-minute bake in an electric oven at approximately 60° Centigrade.

Table IV
Extraction of Air Classified Cottonseed Flours With 80 Percent Isopropanol

Air Classified Flour	Free Gossypol Percent	Total Gossypol Percent
Unextracted [1]	0.060	0.165
Extracted [2]	.004	.085
Unextracted	.210	.280
Extracted	.007	.090
Unextracted	.070	.253
Extracted	.009	.125
Unextracted	.200	.400
Extracted	.012	.173
Unextracted	.120	.335
Extracted	.012	.210
Unextracted	.290	.510
Extracted	.013	.225

[1] Original air classified flour.
[2] Extracted (80% Isopropanol) air classified flour.

Table V
Yield and Analysis of Cottonseed Flour Fractions
Derived From Hexane Extracted Cottonseed Meal

Fraction	Flour Yields Grams	Flour Yields Percent	Free Gossypol Grams	Total Gossypol Percent	Protein Percent	Oil	Volatile Matter
1	927	19.3	0.064	0.308	53.27	4.5	6.10
2	625	12.9	.064	.280	53.31	4.5	7.04
3	448	9.3	.073	.291	53.24	4.5	6.97
4	403	8.4	.104	.396	52.52	4.5	6.00
Composite	2403	49.9	.072	.312	53.09	4.5	6.49
Composite (bleached)			0.020	0.028	54.56	0.5	5.35

Results obtained with 4814 gram sample of meal.

It is of particular importance to note that the total gossypol value was reduced by a factor of ten—0.312 percent to 0.028 percent. This observation would indicate a difference in the bound gossypol (total minus free) of air classified gland-free flours and the bound gossypol of meals prepared by a heat tempering or cooking step applied to flakes prior to oil extraction. Chlorine is not effective in reducing the bound gossypol values of such meal samples.

Chlorine studies also were conducted in solvents other than in hexane and in air medium. This study was considered important because the favorable results obtained with gland-free classified flours suggested the possible application of the chlorine process in reducing the gossypol content of entire solvent extracted meals containing both free gossypol exterior to the glands and in the intact pigment glands of the meal. Tabulations of results of these studies are presented in Table VI. Results indicate the effective reduction by chlorine action of both free and total gossypol in air classified samples devoid or essentially devoid of pigment glands. No such evidence of gossypol reduction is indicated for either the flotation fines, containing fragmented pigment glands, or the whole hexane extracted meal. However, it is to be noted that chlorine is effective in reducing the total gossypol values of products treated with the polar solvent—isopropanol. This fact is pointed out in data presented for the 91 percent alcohol extractions—with and without chlorine. Without chlorine, the total gossypol value obtained was 0.584 percent as compared to 0.228 percent when chlorine was added. Also to be noted is the chlorine treatment in the mixture of hexane, acetone, and water which resulted in a free gossypol value and a total gossypol value which were essentially equal; that is, no binding of gossypol was obtained.

Table VI
Chlorine Action Investigations

Solvent for Chlorine Action	Gossypol Free, Percent	Gossypol Total, Percent
Air Classified Flours		
Hexane	0.020	0.028
99% Isopropanol	.002	.016
91% Isopropanol	.024	.015
Flour (No Chlorine)	.072	.312
Hexane Flotation Flour (Fine Gland Particles)		
Air	0.200	0.343
Hexane	.184	.396
99% Isopropanol	.090	.168
91% Isopropanol	.050	.228
80% Isopropanol	.037	.440
91% Isopropanol (No Chlorine)	.092	.584
Flotation Flour (No Chlorine)	.540	.700
Entire Hexane Meal		
Air	0.720	0.780
Hexane	.604	.760
91% Isopropanol	(.212)	.448
Meal (No Chlorine)	.840	1.232
Hexane-Acetone-Water	.184	.167
Meal (No Chlorine)	.855	1.140

At this point, it is well to comment on the color of chlorine-treated meals obtained in the different solvent media. Direct action of dry chlorine gas on meals in air caused some charring and produced a flour with a black-brown color. The whitest meal was produced in 99 percent isopropanol and the degree of browning increased as the percent of water was increased in the isopropanol. Hexane bleached meals approach the 99 percent isopropanol meals in color.

Much work must be done to establish the merits of a chlorine treatment; namely, chlorine requirements for different gossypol conditions, nutritive value of the chlorine treated meals, mode of action of chlorine, factors which influence mode of chlorine action (such as moisture of meal, meal components soluble in the solvent used for chlorine treatment, particle size of meal), and action of chlorine diluted with air or inert gasses upon meals of different moisture content. Ultimately it is hoped that a whole hexane meal can be processed into a product of both low free and total gossypol content by chlorine treatment. Throughout this presentation, an attempt has been made to refrain from the term "chlorine bleach" and to refer to such terms as "chlorine action" or "chlorine treatment." The term "chlorine bleach" could be appropriate if it is considered in the broad aspects of the end result obtained;

namely, reduction in gossypol content of the meal or flour and reduction or change in color of the meal or flour. The term "chlorine bleach" as applied to wheat flours denotes enhancing the whiteness of the flour and does not carry the added detoxification concept of "chlorine bleach" as applied to cottonseed flours.

Summary and Conclusions

In summarizing the data which have been presented, I would like to consider a unit process employing both air classification and chlorine. The diagrammatic representation of two alternate processes is outlined in Figure 2. Common to the two processes are the raw material (kernels), moisture control of raw kernels, grinding of raw kernels, and solvent extraction to remove oil. Following the solvent extraction step, route 1 indicates a chlorine bleach of the solvent-wet flakes from the extractor followed by desolventizing, meal grinding or mulling and finally air classification to produce 50 to 70 percent of essentially gland-free flour based on the weight of solvent-free meal put to the chlorine treatment. Route 2, first employs desolventizing of the solvent moist meal from the extractor. Dried meal is then ground, air classified, treated with chlorine in solvent and desolventized. Yield of air classified flour would be essentially the same as realized by route 1.

Liquid comminution of pre-extracted flakes, with or without chlorine treatment during comminution, if applicable to final air classification, would represent a simplification in that the meal grinding step could be removed from both routes and a 50 to 70 percent yield of flour could still be realized Chlorine treatment in conjunction with the comminution would further reduce steps required in that the bleached meal fines, collected by filtration or centrifugation, could go directly to desolventizer and then to air classifier. Many other variations could be suggested, but the ultimate success of a particular process must be determined by economic evaluation. Such economic evaluation must be ascertained by processing cost in reference to type of end product desired and the particular use for the end product—for poultry or animal feed or for human consumption.

We wish to acknowledge the assistance afforded by Mr. A. Cecil Wamble and his staff of the Cottonseed Products Research Laboratory at A. & M. College of Texas in the pursuit of this research investigation.

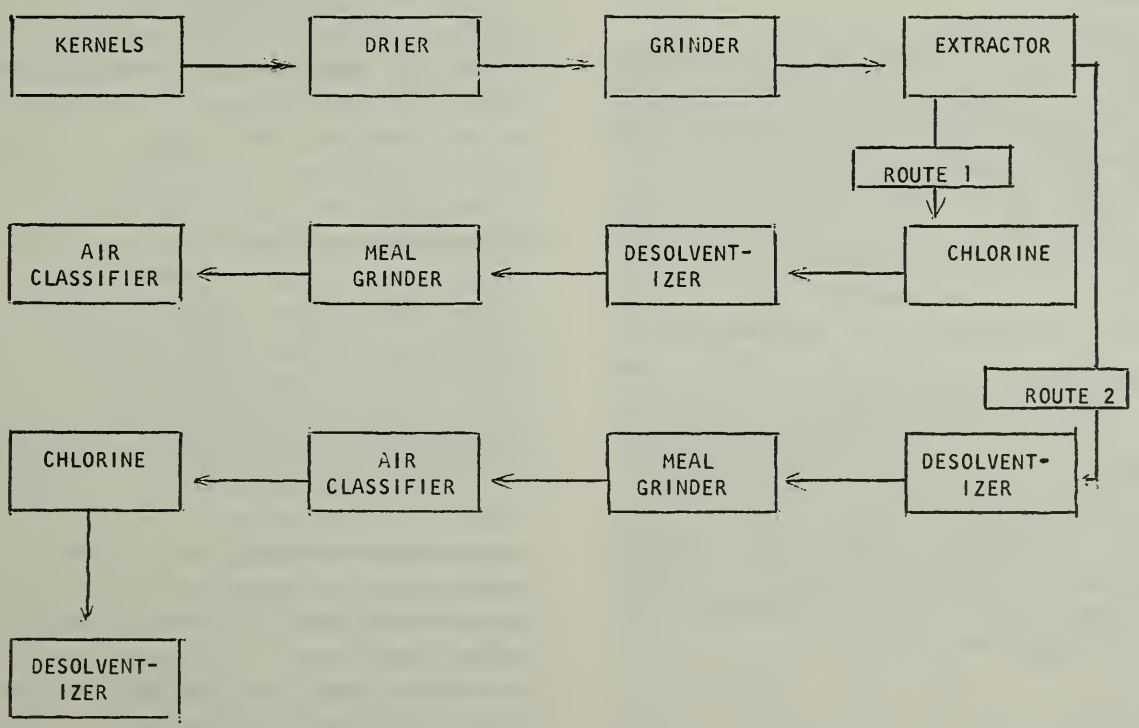

Fig. 2.—Flow sheet for air classification-Chlorine process.

DISCUSSION

Question: Were you able to test for gossypol on your meals and extracts? Did the presence of residual chlorine affect the test?

Meinke: I made an extract of gossypol obtained from raw kernels or hexane-extracted meal, then subjected that to chlorine bleach and different solvents. We got color changes over spectrum — green, brown, purple—but the main effect was that the residual chlorine messed up the gossypol assay at that particular point. In other words, we got browning of the aniline itself. However, in meals which had been air dried as indicated and baked for about 30 minutes in an oven, and let stand for a period of time, there wasn't any indication that there was a change in those particular values. I would have faith in the solid samples but I would be hesitant to make a comment on the extracts if they were assayed for gossypol. As I indicated, this is truly preliminary and the only thing that I attempted was the free and total gossypol values in the meal. We have not investigated the aspects of oil refining.

Question: Is it possible to separate the glands from the meal by some electric phenomena?

Meinke: I can say this: the meal particles will take on a charge in the air classifier and cling to the side of the glass. In that respect, it would be possible to put a charge on them.

A committee selected from the attendees of the Conference held a meeting to discuss guidelines for cottonseed protein products of high nutritive value. The conclusions developed were presented to the Conference. In line with the several discussions, the following tentative quality and processing guide for cottonseed flour for human consumption has been prepared by Dr. Max Milner, Senior Food Technologist, Food Conservation Division, UNICEF.

TENTATIVE QUALITY AND PROCESSING GUIDE COTTONSEED FLOUR FOR HUMAN CONSUMPTION

I. Introductory:

The following description of the processing and quality characteristics for edible cottonseed flour is offered as a general guide for food manufacturers and others interested in WHO/FAO/UNICEF-assisted feeding programs. High nutritive value of the protein and safe levels in the product of toxic or otherwise undesirable components are the paramount objectives of the processing since the food will be a major dietary source of protein for infants and children. This requires special care in all phases of manufacture, including selection of raw material, control of every processing step, sanitation, packaging, storage and handling. Information concerning various critical phases of processing as well as regulating the quality of the finished product will be indicated in the following paragraphs.

II. General:

Useful descriptions of processing techniques for cottonseed oil meal as manufactured primarily for animal feeding are found in monographs by Altschul (Processed Plant Protein Foodstuffs, Ed. by Aaron Altschul, Academic Press, 1958), and Bailey (Cottonseed and Cottonseed Products, Ed. by Alton E. Bailey, Interscience Publishers, Inc., 1948). The objective of the WHO/FAO/UNICEF Protein-rich Foods Program is to upgrade the meal into a flour suitable for human consumption.

The edible flour should be produced from cleaned high-quality cottonseed which by analysis contains not more than 1.0% of foreign matter, including seeds of other species, not more than 12.0% moisture, and not more than 1.8% free fatty acids in the oil of the seed. Badly discolored kernels should not exceed 5%.

Conventional equipment for cottonseed processing may be employed and operated in a manner to yield a product as described. Dehulling will need to be more thorough than normal to attain compositional values in the product suggested in Section III. Only freshly flaked meats are to be used and these should not be subjected to cooking and pressing temp-

eratures exceeding 250° F. If solvents are used, they shall be nonchlorinated, uncontaminated, and acceptable for food processing. Lubricants used should be free of chlorinated compounds.

The plant and process should be kept sanitary, consistent with accepted standards for production of food for human consumption.

III. Analysis:

1. **Composition:**

The finished product should have the following limits for chemical factors, expressed on an "as is" moisture basis, using recognized analytical procedures (Consult: Association of Official Agricultural Chemists, Official Methods of Analysis, 9th Ed., 1960 or Official and Tentative Methods of the American Oil Chemists' Society).

Moisture % (maximum)	10.0
Crude fat % (maximum)	6.0
Protein % (N x 6.25, minimum)	50.0
Crude fiber % (maximum)	5.0
Total gossypol % (maximum)	1.0
Free gossypol % (maximum)	.055
Free fatty acids (maximum, as percent of oil)	1.8
Available lysine, g/16g nitrogen (minimum)[1]	3.6

[1] For method see next paragraph.

2. **Quality Control Methods Suitable for Guidance of Processing:**

(a) **Available Lysine** (Consult: Conkerton, Edith J. and Frampton, V. L. Archives of Biochemistry and Biophysics 81: 130-134, 1959; also Baliga, B. P.; Bayliss, M. E.; and Lyman, C. M. Archives of Biochemistry and Biophysics 84:1-6, 1959).

Note: The methods cited are those most recently published. Extensive study of this methodology is continuing and references to useful new developments will be available through WHO, FAO or UNICEF, as they appear.

In cottonseed flour, a high value for available lysine (also called free epsilon-amino lysine) is desirable since it appears to be correlated positively with protein nutritive value as determined by controlled rat feeding trials.

(b) **Soluble Protein (as percent of total protein)** (Consult: Lyman, C. M.; Change, W. Y.; and Couch, J. R. J. Nutrition 49: 679-690, 1953).

This method is frequently utilized to control the heat treatment step of processing and provides an index of loss of nutritive value of the protein with processing. A minimum value of 65% is recommended.

3. **Sanitary Analyses**

(a) **Bacteriology**. The total bacterial plate count should not be more than 10,000 per gram when assayed by the methods of the American Public Health Association or equivalent accepted methods. Analysis of a representative sample of the flour should show no **Escherichia coli** and no salmonellae to be present. The raw flour should be safe for human consumption.

(b) **Acid Insoluble Ash** (Consult: Woodman, A. G. Food Analysis. McGraw Hill, 1941, p-26). This determination, carried out on the ash obtained from the product, is expressed as percentage of the original sample, dry weight basis, and indicates contamination with extraneous mineral matter such as sand and dirt. The value in any event should not exceed 0.1%.

(c) **Insect and Rodent Contamination** (Consult: Association of Official Agricultural Chemists, Official Methods of Analysis, 9th Ed. 1960, para. 35.40). Edible cottonseed meal or flour should be essentially free of insects, insect fragments, rodent hairs, and rodent excreta as determined by the method indicated.

IV. Additives:

Sodium propionate, as a preservative (fungal inhibiter) may be added at the rate of 0.3% by weight, maximum.

Grinding and sieving, or classifying can be used to provide products of mesh sizes as desired. They may be packed in locally accepted quantity units in such materials as multi-wall Kraft bags having polyethylene or similar liners which will not contribute to contamination and which will maintain the product in storage and transport in a dry sanitary condition. For sea shipment, such bags should be provided with jute over-wrapping.

SUMMARY OF CONFERENCE AND COMMENTS

by
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We shall attempt to review and comment on the factors which influence the quality of cottonseed protein products, on methods for measuring quality, and on some perspectives for further research.

Factors Which Influence Quality

(a) **Genetic:** First and foremost in determining the nature of cottonseed products are the genetics factors. These determine the kind and amounts of the various constituents; both the type and range in quantities of protein and oil content of cottonseed are genetically determined. Within a given variety there is generally little variation in the amino acid composition of the protein, as was shown for a wide selection of soya beans. While it has been possible to breed more protein into corn, this has resulted mostly in an increase in one fraction, zein fraction. The genetically determined factors, therefore, set the limits of what can be expected from any given seed source.

But genetics need not be a static factor. A striking example is the work that is now going on to produce glandless cottonseed. The report of Dr. Barker was interesting and optimistic, but, as he has pointed out, 85% of the value of the seed is in the lint; therefore, breeding specifically for a seed characteristic must, above all, maintain or improve the lint characteristic if it is to become a commercially interesting venture. It will also be of interest to know whether there are any other major changes in the composition of cottonseed when it is converted to a glandless variety.

(b) **Environment, Storage, and Handling Factors:** Even though the range of composition and the nature of the materials are determined by genetic factors, within this range there are wide variations in protein, oil, and gossypol content depending upon location of growth and the kind of growing season. A number of these variations were pointed out by Mr. Hopper; it is possible to reduce considerably the gossypol content of seeds by selection of seeds from certain growing areas.

Individual seasonal variations may influence profoundly the amount and quality of the oil and also of the protein.

Then there is storage damage. Cottonseed is sensitive to storage damage for mechanical reasons if for no other reasons. Because of the lint on the seed, it cannot be moved automatically. Whereas the clean seed like flax, rice, or soybean can be turned from the bottom to the top of an elevator automatically to prevent heating, this cannot be done for cottonseed. Moreover, the lint is a good insulating agent promoting heating during storage. Therefore, if damaged seed or seed of higher than normal moisture content are put into storage, there is a good possibility that it will spoil. This will be reflected in a lowering in the quality of the oil and no doubt in the protein too. Products which are designated for the highest quality outlets, therefore must be produced from prime seed which has not suffered any damage either during the growing season or on storage.

(c) **Processing:** Considerable control can be exercised on the quality of cottonseed products by the methods used for processing, For example, the amount of oil left in the cake is a processing variable. If one uses an inefficient process such as the hydraulic press, 6% or more oil is left in the cake. An efficient process such as solvent extraction or some pressing combination which finally involves solvent extraction will leave less than 1% in the cake.

Fiber content is another controllable variable depending entirely on the degree of cleaning of the seed prior to processing and on the amount of hull removal in the final product.

Since oil and fiber content determine the protein content, then protein content within the limits of the genetic factors and of any particular season can be controlled by the degree of oil- and fiber-removal. The amount

of protein in the final product, therefore, is controllable and depends on the choice of the operator who for economic reasons will either garner the oil or the protein.

The removal of gossypol is in part a processing variable. The reduction in the amount of free gossypol in the meal is certainly a function of the processing condition. One can produce meals and flours with high concentrations of free gossypol if the oil is extracted directly with hexane. But if the oil is removed by screw pressing or by screw pressing followed by solvent extraction, low concentrations of free gossypol are the results. The hydraulic press process which involves a cooking step will produce products with intermediate contents of free gossypol.

Total gossypol is not clearly a processing variable. It is possible by screw pressing with the minimum amount of cooking to expel the larger amount of the gossypol into the oil from which it can be removed by refining. In this case, there is a reduction in total gossypol by processing. The process described by Mr. Hopper and Dr. Frampton of azeotropic extraction of cottonseed certainly, if it were successful in its other objectives, would make the reduction of free and total gossypol a processing variable.

The process mentioned by Dr. Meinke is interesting. I believe that he realizes, of course, that the introduction of chlorine into a food material is a process that might properly be suspect. Some of the most toxic compounds that have turned up lately have been chlorinated hydrocarbons. Dr. Cowan especially knows this since he was among those who discovered the particular compound—a chlorinated derivative of sulfur compound of soybeans—which is very toxic and which has been responsible for the difficulties with soybean meals prepared by extraction with chlorinated solvents.

Heat is a processing variable; the beneficial or detrimental effects of heat are in part a processing variable. Heating can be completely eliminated by the azeotrope process of Dr. Frampton. It can be minimized in screw pressing by lowering the temperatures of cooking prior to pressing and by not using any more pressing than is necessary to attain the processing objectives. If minimum heat damage to the protein is the most desirable objective, then, as Dr. Lyman pointed out, it may be desirable to leave a higher oil content in the meal in order to reduce the amount of work required to process the material.

It may be that some heat is advantageous. Dr. Lyman presented interesting preliminary experiments on the wet cooking of cottonseed which did not damage the material and perhaps even improved a little the lysine availability and nutritive value.

Changing the amino acid pattern may be considered a processing variable. Supplementation with amino acids as was discussed by Dr. Phelps could, in some instances, be very useful as a means for improving the nutritive value of the supplement protein. Dr. Allison raised an interesting point when, in discussing gluten, he pointed out that there is a limit to improvement by supplementation of gluten with amino acids because the relative proportion of essential to nonessential amino acids was too low. In many of the seed proteins, there is an enormous amount of amides and in some of the arginine. It is entirely possible that ways may be found to reduce the amount of non-essential amino acids in the material, and this might improve the quality of protein.

Measurement of Quality of Cottonseed Protein

(a) **Composition:** The primary measurement of cottonseed quality is its composition of oil, fiber, protein, and moisture. These measurements are indispensable for definition of the materials. For if there is not enough protein to begin with (if the fiber and oil removal are not sufficiently complete), then quality consideration is secondary.

(b) **Available lysine:** Available lysine seems to be the best available measure for the extent of heat damage during the processing. Dr. Frampton has reported correlation between available lysine and nutritional performance of cottonseed protein in animal experiments. This view is supported by Dr. Mauron who showed that for peanut products and milk powder, available lysine was an important measurement. It is interesting to note from the data of Dr. Frampton that total lysine is rather easily reduced during processing. But the reductions in total lysine content are not quite as great as those for available lysine. Therefore, there seems to be an advantage to using available lysine over total lysine as a measure of the quality of protein.

(c) **Gossypol Content:** The meaning of gossypol content is becoming more and more

difficult to assess. There is no question that gossypol is a toxic material. Dr. Edward Eagle many years ago proved that gossypol is toxic; but the question remains as to the relative importance of free and total gossypol and whether there are other materials in the meals which are interfering with the protein. Lyman, several years ago, reported that the protein level in the diet had an influence on the toxicity of gossypol. In experiments on swine which are very sensitive to gossypol, he showed that on a level of 13% protein in the diet there were a number of deaths, but when the same meal was used at 18% protein in the diet there were no deaths at all. This would suggest an interrelationship between protein content, and probably protein quality, and gossypol effects.

The relative role of free and total gossypol is also difficult to assess. According to Dr. Frampton, variations in free gossypol at low levels of free gossypol did not seem to have any effect in feeding experiments; the quantity of total gossypol had more effect. But the question remains whether total gossypol is not inversely related to available lysine.

(d) **Description of Processing Conditions:** When it is not possible to isolate all of the chemical factors which determine the quality of a source of protein, it is sometimes a good idea to include in a description of material a description of its processing conditions. Ultimately when all of the pertinent chemical measurements are known, such a description is no longer needed. By screw press processing, it is more easily possible to manufacture a meal with low free gossypol and low heat damage than by hydraulic pressing. Direct solvent extraction will not reduce the gossypol content. Therefore, a knowledge of the processing condition will clarify and lend another dimension to the analytical results.

(e) **Nitrogen Solubility:** The importance and meaning of nitrogen solubility is not clear. There are some who feel—and among them myself—that nitrogen solubility was at one time a useful approach. Now that there is the measure of available lysine which correlates well with nutritional performance, it might well be that there is no further need for solubility measurements. There are others who feel that it still has not lost its usefulness, particularly as a control measurement in a plant.

Perspectives in Research

Those of you who perhaps have been brought in contact with the cottonseed picture for the first time might well be impressed with the enormous amount of information available on cottonseed as a source of protein for animals and man. I venture to guess that perhaps it is presently one of the best studied of oilseeds. But, because there is so much information available, there are conflicting reports and there are a number of unanswered questions, particularly on the role of gossypol in the behavior of cottonseed products. Some of the answers to these questions will come out of intensification and continuation of the same kind of research that has been undertaken in the past; but it may be entirely possible that some new approaches might be needed to obtain some of the information that is lacking. We might characterize the present approach to cottonseed as the Food Technology approach. By this, I mean a research in which one living material is converted to suit nutritive ends of man or man's animals. The material in question is investigated from the point of view of its suitability for use by man or his animals and from the point of view of conversions to such use. This type of research has brought about outstanding benefits to society. After all, all of the food that we now have and so many of the new innovations in food which have made living richer and more economical have come about as a result of advances in Food Technology.

But there is a limit to what can be done in understanding the composition of a seed from the point of view of Food Technology because the questions that Food Technology asks are of a special sort. The proteins of a seed, for example, have been studied from the nutritional point of view, not from the role which they play in the seeds themselves. The lipids are studied as factors in diet, not from the point of view of the seed economy. It is entirely possible that new and vital information on the protein, lipids, and the minor components could be obtained more easily or perhaps only by studying the seed as a botanical material. This is the point of view that we have taken in the Seed Protein Laboratory. If we want to know more about the proteins of the seed, we must understand them in terms of their function in the seed. We must learn, for example, to dis-

tinguish between reserve proteins and functional proteins on the basis of changes these proteins undergo on germination. It is only in the activities of the seed itself that these proteins have a real meaning. And once a biological handle is obtained, then these proteins can be purified, isolated and studied, and information vital not only to answer botanical questions but also to answer nutritional questions would be the result.

We might, therefore, expect that a combination of the two types of research, Food Technology and Biochemistry, will provide suggestions for new types of approaches to making the protein of cottonseed more available to man and his animals.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

Program for

CONFERENCE ON COTTONSEED PROTEIN FOR ANIMAL AND MAN

November 14-16, 1960

SOUTHERN REGIONAL RESEARCH LABORATORY
1100 ROBERT E. LEE BOULEVARD
NEW ORLEANS, LOUISIANA

Honorary Chairman: A. L. Ward
National Cottonseed Products Association

Planning Committee:
Aaron M. Altschul
Chairman

Layton E. Allen
T. H. Hopper
Ralph M. Persell
Richard E. Phelps
E. L. Patton

Monday, November 14, 1960—1:00 p. m.

INTRODUCTORY REMARKS (A. L. Ward, Chairman)

C. H. Fisher, Director, Southern Utilization Research and Development Division

Layton E. Allen, Senior Engineer, Food Conservation Division, UNICEF

Garlon A. Harper, Director, Research and Educational Division, National Cottonseed Products Association

NUTRITION (Philip D. Aines, Chairman)

"Nutritive Value of Cottonseed Protein for Monogastric Animals." James B. Allison, Director, Biological Research, Rutgers University, New Brunswick, N. J.

"Nutritive Value of Cottonseed Combinations in Rat and Chick Biological Trials." Ricardo Bressani, Chief, Division of Agricultural and Food Chemistry, Instituto de Nutricion de Centro America y Panama, Guatemala, C. A.

"Nutritive Value of Cottonseed Protein for Humans." Nevin S. Scrimshaw, Director, Instituto de Nutricion de Centro America y Panama, Guatemala, C. A.

"Experiences with Cottonseed Flour in Peru." R. B. Bradfield, International Cooperation Administration, Washington, D. C.

"Supplementation of Cottonseed Protein with Lysine." Richard E. Phelps, Assistant Director, Research and Education Division, National Cottonseed Products Association.

5:00 p. m.—Adjourn

Tuesday, November 15, 1960—9:15 a. m.

REVIEW OF RELATIONSHIP BETWEEN COTTONSEED CONSTITUENTS AND PROTEIN VALUE (Garlon A. Harper, Chairman)

"Breeding of Gossypol-Free Cottonseed: A Progress Report." H. D. Barker, Chief, Cotton and Cordage Fiber Research Branch, Agricultural Research Service, Beltsville, Md.

"Review of Epsilon-amino Groups as a Measure of Available Lysine." Jean Mauron, Nestle Company, Vevey, Switzerland.

"Epsilon-amino Lysine." Vernon L. Frampton, Industrial Crops Laboratory, Southern Utilization Research and Development Division, New Orleans, La.

"Gossypol." Carl M. Lyman, Head, Department of Biochemistry and Nutrition, Texas A. & M. College, College Station, Texas.

"Lipids." A. R. Kemmerer, Head, Department of Agricultural Biochemistry, University of Arizona, Tucson, Ariz.

12:00 Noon—Box Lunch, Lobby

Tuesday, November 15, 1960—1:00 p. m.

PREPARATION OF COTTONSEED PROTEIN PRODUCTS (E. L. Patton, Chairman)

"Developments in Preparation of Industrial Cottonseed Protein Products." H. D. Fincher, Anderson, Clayton and Company, Houston, Tex.

Discussion—Vernon L. Frampton

"New Developments at SU on Cottonseed Products of High Nutritive Value." T. H. Hopper, Chief, Industrial Crops Laboratory, Southern Utilization Research and Development Division, New Orleans, La.

"New Developments at Texas A. & M. College on Cottonseed Products of High Nutritive Value." W. W. Meinke, Manager, Chemurgic Research Laboratory, Texas Engineering Experiment Station, College Station, Tex.

5:00 p. m.—Adjourn

Wednesday, November 16, 1960—9:00 a. m.

GUIDELINES FOR COTTONSEED PROTEIN PRODUCTS (A. M. Altschul, Chairman)

Discussion of Guidelines for Cottonseed Protein Products of High Nutritive Value.
Summary.

12:00 Noon—Adjourn

ATTENDANCE LIST

"COTTONSEED PROTEIN FOR ANIMAL AND MAN"
November 14-16, 1960

Philip D. Aines, The Procter & Gamble Co., Food Division, Box 201, Cincinnati 24, Ohio

Layton E. Allen, Senior Engineer, Food Conservation Division, UNICEF, United Nations, New York

James B. Allison, Director, Bureau of Biological Research, Rutgers, The State University, New Brunswick, New Jersey

W. B. Anthony, Professor & Nutritionist, Animal Husbandry & Nutrition, Auburn University, Auburn, Alabama

H. D. Barker, Chief, Cotton & Cordage Fiber Research Branch, Crops Research Division, ARS, U. S. Dept. of Agriculture, Beltsville, Maryland

Ben F. Barrentine, Head, Department of Chemistry, Mississippi Agricultural Experiment Station, State College, Mississippi

R. B. Bradfield, International Cooperation Administration, U. S. Operations Mission, U. S. Embassy, Lima, Peru

Lewis Branscombe, Gold-Kist Peanut Growers, Graceville, Florida

J. H. Brawner, Chief Engineer, Southern Cotton Oil Division, Wesson Oil and Snowdrift Co., 210 Baronne Street, New Orleans 12, Louisiana

Ricardo Bressani, Chief, Division of Agricultural & Food Chemistry, Instituto de Nutricion de Centro America y Panama, Oficina Sanitaria Panamericana, Sector of Centro Panamericana, Carretera Roosevelt, Zone 11, Guatemala, C. A.

H. A. Campbell, General Foods Corporation, 555 S. Broadway, Tarrytown, New York

J. C. Cowan, Chief, Oilseed Crops Research Laboratory, Northern Utilization Research & Development Division, Peoria, Illinois.

L. V. Curtin, Assistant Director, Feed Research & Nutrition, McMillen Feed Mills, Decatur, Indiana.

H. D. Fincher, Anderson, Clayton & Company Houston, Texas

W. R. Graham, Director of Research, The Quaker Oats Co., Banington, Ill.

Herberto Gutierrez, Anderson, Clayton & Company, Mexico City, Mexico

Garlon A. Harper, Director, National Cottonseed Products Association, Inc., Research & Educational Division, 618 Wilson Bldg., Dallas 1, Texas

L. A. Hewgill, Vegetable Oil Extraction Co., Baghdad, Iraq

H. M. Hind, Gold-Kist Peanut Growers, Graceville, Florida

John Hopper, Director of Nutrition, Kellogg Co., Battle Creek, Michigan

M. L. Karon, Standard Fruit and Steamship Co., New Orleans, Louisiana

A. R. Kemmerer, Head, Department of Agricultural Biochemistry, University of Arizona, Tucson, Arizona

L. E. Kovacs, President, Vitamins, Inc., 809 W. 58th St., Chicago 21, Illinois

Bernardo Lopez, General Manager, P.A.S.A. San Salvador, El Salvador, C. A.

Carl M. Lyman, Head, Department of Biochemistry and Nutrition, Texas A. & M. College Station, Texas

Robert V. Mac Allister, Laboratory Director, Research Center, General Foods Corp., Tarrytown, New York

Jean Mauron, c/o National Institute of Dental Research, Laboratory of Biochemistry, National Institutes of Health, Bethesda 14, Maryland, (Nestle Co., Vevey, Switzerland)

W. W. Meinke, Manager, Chemurgic Research Laboratory, Texas Engineering Experiment Station, P. O. Box 221, College Station, Texas

Max Milner, UNICEF, United Nations, New York

Daniel L. Noyes, Project Development Manager, Exports & Latin American Operations, Quaker Oats Co., 120 Wall St., New York 5, N. Y.

Giannetto Paggi, H. de Sola e Hijos, San Salvador, El Salvador, C. A.

Richard A. Phelps, Assistant Director, Research & Educational Division, National Cottonseed Products Association, 618 Wilson Bldg., Dallas 1, Texas

W. G. Quinn, Manager, Technical Division, The Buckeye Cellulose Corp., Cincinnati 17, Ohio

Nevin S. Scrimshaw, Director, Instituto de Nutricion de Centro America y Panama, Oficina Sanitaria, Panamericana, Carretera Roosevelt, Zona 11, Guatemala, C. A.

Frank H. Smith, Research Associate Professor, N. C. State College, School of Agriculture, Raleigh, N. C.

A. L. Ward, c/o National Cottonseed Products Association, Dallas 1, Texas

A. B. Watts, Head, Poultry Industry Department, A.E.S., Louisiana State University, Baton Rouge 3, Louisiana

Richard C. Whittington, 3706 Norfolk, Houston, Texas

Harold L. Wilcke, Asst. Director of Research, Ralston Purina Co., St. Louis, Missouri

Walter J. Wolf, Northern Utilization Research and Development Division, Peoria, Illinois

Madelyn Womack, Biochemist, Human Nutrition Research Division, U.S.D.A., Washington, D. C.

ATTENDANCE LIST

on

Participants of Southern Division in

COTTONSEED PROTEIN FOR ANIMAL AND MAN CONFERENCE

November 14-16, 1960

* * * *

G. E. Goheen, Acting Director of Division

R. M. Persell, Assistant Director

T. H. Swan, Assistant to Director

A. M. Altschul, Chief Research Chemist, Seed Protein Pioneering Research Laboratory

T. H. Hopper, Chief, Industrial Crops Laboratory

V. L. Frampton, Head, Oilseed Meals Investigations, Industrial Crops Laboratory

E. L. Patton, Chief, Engineering and Development Laboratory

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
NEW ORLEANS, LA.

FOR IMMEDIATE RELEASE September 26, 1960

Cottonseed Protein In Human and Animal Nutrition Subject of November Conference:

"Cottonseed Protein for Animal and Man" will be the subject of a conference Nov. 14-16 at the U. S. Department of Agriculture's Southern Utilization Research and Development Division in New Orleans, La.

Sponsors of the conference are the United Nation's Children's Fund (UNICEF), the National Cottonseed Products Association, and the U. S. Department of Agriculture.

A planning committee composed of A. M. Altschul, of the Southern Division, chairman; Layton E. Allen, of the Food Conservation Division, UNICEF; Richard Phelps, National Cottonseed Products Association, Dallas; and V. L. Frampton, T. H. Hopper, E. L. Patton, and R. M. Persell, all of the Southern Division, met Sept. 9 in New Orleans to work out details of the conference.

Purposes of the conference, as outlined by the planning committee, will be:

(1) To review the available information on cottonseed protein in rations for animal and man;

(2) To define optimum quality of cottonseed products;

(3) To consider feasible methods and conditions for producing cottonseed protein of high nutritive quality; and

(4) To pinpoint areas of research necessary to foster broader use of cottonseed protein.

Those who have already accepted invitations to participate in the program are: J. B. Allison, Director of the Bureau of Biological Research, Rutgers University, New Brunswick, N. J.; H. D. Fincher, Anderson-Clayton & Co., Houston, Texas; V. L. Frampton, Southern Division, New Orleans; A. R. Kemmerer, Head, Department of Agricultural Biochemistry, University of Arizona, Tucson; C. M. Lyman, Department of Biochemistry and Nutrition, Texas A&M College, Bryan, Texas; Jean Mauron, Nestle Co., Vevey, Switzerland; and Nevin S. Scrimshaw, Instituto Nutricion de Centro America y Panama, (INCAP) Guatemala.

Program for the meeting is as follows:

Monday, 1 p. m.

1. Nutritive Value of Cottonseed Protein for Monogastric Animals
2. Nutritive Value of Cottonseed Protein for Humans
3. Supplementation of Gossypol Protein with Lysine

Tuesday, 9 a. m.

4. Breeding of Gossypol-free Cottonseed: A Progress Report
5. Review of Epsilon-Amino Groups as a Measure of Available Lysine
6. Review of Relationship between Cottonseed Constituents and Protein Value
   a. Epsilon-Amino Lysine
   b. Gossypol

Tuesday, 1 p. m.

Review of Relationship between Cottonseed Constituents and Protein Value (continued from morning session)

   c. Lipids

7. Preparation of Cottonseed Protein Products

Wednesday, 9 a. m.

8. Discussion of Guidelines for Cottonseed Protein Products

12 Noon—Adjournment

A committee will be named to evaluate available information and outline standards to be used as a guide for the preparation of cottonseed protein products for human consumption. This committee will report findings Wednesday morning to the general conference for discussion. The remainder of the Wednesday morning session will be devoted to a discussion of the status of research on cottonseed protein.

The conference was proposed as a result of UNICEF interest in the use of cottonseed protein as a dietary supplement in areas of the world where the supply of animal protein is inadequate. It is already being used for this purpose to some extent.

Anyone interested in attending should write to T. H. Swan, Southern Utilization Research and Development Division, Box 19687, New Orleans 19, La.

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
NEW ORLEANS, LA.

FOR IMMEDIATE RELEASE December 9, 1960

Cottonseed Flour as Source of Protein for Human Nutrition is Conference Subject:

An international conference of great importance to human welfare throughout the world took place at USDA's Southern Utilization Research and Development Division in New Orleans, La., recently. This was the Conference on Cottonseed Protein for Animal and Man, held in relation to the use of cottonseed flour as a source of protein in cotton-producing areas of the world where supplies of animal proteins for the human diet are insufficient. The conference was sponsored jointly by UNICEF, the National Cottonseed Products Association, and the Southern Division. Dr. A. M. Altschul, Southern Division, served as general chairman for the conference, and A. L. Ward, retired Director, Research and Educational Division, NCPA, was honorary chairman.

The primary objective of the conference was to develop standards of quality for use as guidelines in the production of cottonseed flour for this purpose in the light of new knowledge about cottonseed and cottonseed processing accumulated within the past few years. With approximately 50 participants from industry and various research agencies in this country and abroad, the entire field was covered exhaustively in the two days of presentations and discussions. Representatives of UNICEF, which is sponsoring the use of plant proteins for improvement of the human diet, were Layton E. Allen, Senior Engineer, Food Conservation Division, and Max Milner, both of United Nations, New York.

Dr. Nevin S. Scrimshaw, Director, Instituto de Nutricion de Centro America y Panama, Guatemala, C. A., and Dr. R. B. Bradfield, International Cooperation Administration, U. S. Operations Mission, Lima, Peru, reported on results of the use of cottonseed flour among children of Guatemala and Peru. Both stressed the effectiveness of the cottonseed flour mixtures in preventing or treating kwashiorkor, the disease brought on by severe protein deficiencies. Dr. Scrimshaw cited examples which indicated that at normal and high levels of intake children did as well on the vegetable protein mixture as on milk. Dr. Bradfield said that the vegetable protein mixture was accepted very well in Peru, and is used in the preparation of a variety of native dishes. Dr. Scrimshaw underlined the need for an effective vegetable protein supplement by saying that the death rate among children under one year of age in Guatemala is 40 times that of the United States; of these deaths, about one-third are directly due to kwashiorkor, one-third to childhood diseases, such as whooping cough, whose seriousness is aggravated by the protein-deficient diet, and the other third to diarrhea and other ailments brought on by such deficiencies.

Nutritive quality of cottonseed protein for the nutrition of nonruminant animals was shown to be high according to reports of biological trials by Dr. Ricardo Bressani, Chief, Division of Agricultural and Food Chemistry, Instituto de Nutricion Centro America y Panama. Discussing results, Dr. Bressani said that according to biological results with the cottonseed flour, nutritive value is higher than would be expected from the chemical analysis.

It was reported that Incaparina, the formulation developed by INCAP (Instituto de Nutricion de Centro America y Panama) in clinical trials with a number of children, proved to be as effective as milk protein in promoting health and growth. This formulation consists of cottonseed flour, corn flour, sorghum, dehydrated leaf meal, and torula yeast.

Similar formulations, made up of cottonseed flour, yeast, and various native plant products, have given good results in tests in Peru, Dr. Bradfield said. The mixtures were well received in various acceptability tests, and Peruvians using them have suggested a number of different dishes in which the supplement can be incorporated.

Dr. James B. Allison, Rutgers University, New Brunswick, N. J., comparing the nutritive value of cottonseed protein with other dietary protein sources, said that if cottonseed protein is fed in sufficient quantity to produce optimum development in rats, results are similar

to those produced by casein, or milk protein.

The growth rate of animals fed cottonseed meal is closely correlated with the amount of lysine present in the meal, according to Dr. V. L. Frampton, of the Southern Division, Gossypol did not show any measurable effects on growth rate, but he emphasized this does not mean it does not have harmful effects. The results reported were based on cooperative feeding tests involving some 65 different cottonseed meals, and about 15,000 animals, including broilers, swine, and protein-depleted rats.

The test for lysine developed by Dr. Frampton and his group was acknowledged by the conference to be the most practical and accurate yet devised.

Methods of preparing cottonseed protein products were discussed by H. D. Fincher of Anderson, Clayton & Co., Houston, who said that flotation is a practical method for separating the fine flour from coarser and heavier materials. While it has been recommended that material rejected through air separation should be diverted to other uses, and not recycled to the grinder, he suggested that where pure meats are processed, it appears that a major portion of the material can be recovered as flour without any serious reduction in protein content.

Dr. C. M. Lyman, of Texas A&M College, told the conference that heating cottonseed in an excess of water does not reduce the protein value of the meal, and may even improve it.

Dr. Richard A. Phelps, Assistant Director, Research and Educational Division, NCPA, presented data on the feeding of rats, chicks, and pigs to show that cottonseed meal with added lysine gave much better results than unsupplemented cottonseed meal. Recent information, he said, lends further support to the belief that wherever poor growth in nonruminants was observed, it was due to inadequate consumption and a deficiency of lysine, rather than to any "toxic" manifestation of gossypol. Pigs fed cottonseed meals supplemented with 0.3% L-lysine gained 60% more than those receiving the unsupplemented meal. In other tests, gains varied 30% to 113% when L-lysine was added to the meal. Similar results were obtained with chicks and rats. The animals also consumed larger amounts of feed when lysine was added.

Need for rapid, accurate analytical methods to measure the nutritive value of proteins was emphasized by Dr. Jean Mauron, of the Nestle Co., Vevey, Switzerland, who said the first of the amino acids to be altered by heat during processing is lysine. Accurate determination of lysine in a protein before and after processing should therefore give an evaluation of heat damage to proteins. He pointed out that usual lysine determination after acid hydrolysis does not give the true amount of lysine actually available in the heated protein, but the measurement of epsilon amino lysine more closely reflected the available lysine. He described another method, using digestive enzymes which gave results correlating closely with biological tests.

Small amounts of propene ring fatty acids in cottonseed meal may exert appreciable effects on the nutritive value of the meal, A. B. Kemmerer, of the Arizona Agricultural Experiment Station, told the conference. He said that compounds containing these acids fed to laying hens increased the tendency toward discoloration of the eggs, and small quantities also decreased the hatchability.

A new method of extraction with an acetone-hexane-water mixture to produce cottonseed meal with a low gossypol content and high nutritive value was described by T. H. Hopper, of the Southern Division.

Other speakers and their subjects were: Dr. H. D. Barker, Chief, Cotton and Cordage Fiber Research Branch, Agricultural Research Service, Beltsville, Md., "Breeding of Gossypol-Free Cottonseed: A Progress Report"; Dr. Carl M. Lyman, Head, Department of Biochemistry and Nutrition, Texas A&M College, College Station, Texas, "Gossypol"; H. D. Fincher, Anderson, Clayton & Co., Houston, Texas, "Developments in Preparation of Industrial Cottonseed Protein Products"; and Dr. W. W. Meinke, Manager, Chemurgic Laboratory, Texas Engineering Experiment Station, College Station, Texas, "New Developments at Texas A&M College on Cottonseed Products of High Nutritive Value."

These Proceedings were compiled and edited for

AGRICULTURAL RESEARCH SERVICE

by

Thomas H. Swan and Aaron M. Altschul

SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

New Orleans, La.

Growth Through Agricultural Progress